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METHODS OF SAMPLING AND ANALYSIS FOR SUGAR CONFECTIONERY

(First Revision)

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BUREAU OF INDIAN STANDARDS
MANAK BHAVAN, 9 BAHADUR SHAH ZAFAR MARG
NEW DELHI 110002

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Indian Standard

METHODS OF SAMPLING AND ANALYSIS FOR SUGAR CONFECTIONERY

(First Revision)

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AMENDMENT NO. 1 MAY 2002
TO
IS 6287 : 1985 METHODS OF SAMPLING AND ANALYSIS FOR SUGAR CONFECTIONERY

(First Revision)

(*Page* 13, *clause* **8.3.1**, *line* 6) — Substitute '*m*' *for* '*M*'.

(*Page* 13, *clause* **8.3.2**) — *Insert* '(R)' *after* 'Reducing sugars, percent by mass'.

(*Page* 15, *clause* **9.3**) — Substitute the following for the existing:

'**9.3 Calculation**

9.3.1 *See* **8.3.1** *and* **8.3.1.1**.

9.3.2 Reducing Sugar $(R_1) = \frac{m_1}{M_1} \times 10$

where

m_1 = milligrams of anhydrous dextrose in 1 ml of solution (*see* **9.3.1**), and

M_1 = mass in g of the prepared sample used for making 100 ml of final solution (*see* **9.2**).

9.3.3 Sucrose, percent by mass = $(R_1 - R) \times 0.95$

where

R_1 = reducing sugar obtained after inversion, and

R = reducing sugar originally present (*see* **8.3.2**).

(FAD 15)

Reprography Unit, BIS, New Delhi, India

Indian Standard

METHODS OF SAMPLING AND ANALYSIS FOR SUGAR CONFECTIONERY

(First Revision)

0. FOREWORD

0.1 This Indian Standard (First Revision) was adopted by the Indian Standards Institution on 31 May 1985, after the draft finalized by the Bakery and Confectionery Industry Sectional Committee had been approved by the Agricultural and Food Products Division Council.

0.2 Confectionery products are consumed by children in large quantities. It is, therefore, necessary that strict quality control measures are adopted in their production. This standard was prepared with a view to providing authentic methods of sampling and analysis, and facilitating the interpretation of results on a uniform basis.

0.3 This standard was first published in 1971. It is being revised to take into account the new developments in test methods. For the determination of trace metals the atomic absorption spectrophotometric methods have also been introduced. The method for determination of fat has also been replaced as the earlier one was not suitable for confectionery products.

0.4 In reporting the results of a test or analysis made in accordance with this standard, if the final value, observed or calculated, is to be rounded off, it shall be done in accordance with IS : 2-1960*.

1. SCOPE

1.1 This standard prescribes the methods of sampling and analysis for sugar confectionery.

2. QUALITY OF REAGENTS

2.1 Unless specified otherwise, analytical grade reagents and distilled water (*see* IS : 1070-1977†) shall be employed in tests.

*Rules for rounding off numerical values (*revised*).

†Specification for water for general laboratory use (*second revision*).

3. SAMPLING

3.1 General Requirements of Sampling

3.1.0 In drawing, preparing, storing and handling samples, the following precautions and directions shall be observed.

3.1.1 Samples shall be taken in a protected place not exposed to damp air, dust or soot.

3.1.2 The sampling instruments shall be clean and dry when used.

3.1.3 Precautions shall be taken to protect the samples, the material being sampled, the sampling instrument and the containers of samples from adventitious contamination.

3.1.4 The samples shall be placed in clean and dry glass containers. The sample containers shall be of such a size that they are almost completely filled by the sample. The samples shall be filled loose and not pressed in the container.

3.1.5 Each container shall be sealed air-tight after filling and marked with full details of sampling, date of sampling, batch or code number, name of the manufacturer and other important particulars of the consignment.

3.1.6 Samples shall be stored in such a manner that the temperature of the material does not vary unduly from the normal temperature.

3.2 Scale of Sampling

3.2.1 *Lot* — All the containers in a single consignment of the material drawn from a single batch of manufacture shall constitute a lot. If the consignment is declared to consist of different batches of manufacture, the batches shall be marked separately and the groups of containers in each shall constitute separate lots.

3.2.1.1 Samples shall be tested for each lot for ascertaining its conformity to the requirements of the corresponding specification.

3.2.2 The number of containers to be sampled from each lot shall depend on the size of the lot and it shall be done according to Table 1.

3.2.3 The containers shall be selected at random from the lot and for this purpose a random number table* as agreed to between the purchaser and the supplier shall be used. If such a table is not available, the following procedure shall be adopted:

Starting from any container in the lot count them as 1, 2, 3, ... up to r in a systematic manner, where r is the integral part of $\frac{N}{n}$, N

*See IS : 4905-1968 Methods for random sampling.

being the total number of containers in the lot, n the number of containers to be selected (*see* Table 1). Every rth container thus counted shall be separated until the requisite number n container is obtained from the lot to give the sample for the test.

TABLE 1 NUMBER OF CONTAINERS TO BE SELECTED FOR SAMPLING

(*Clause* 3.2.2)

Lot Size	Number of Containers to be Selected (n) for Size of the Containers	
	500 g and Above	Less than 500 g
N		
(1)	(2)	(3)
Up to 25	3	6
26 to 100	4	6
101 to 300	5	9
301 to 500	7	13
501 and above	9	15

3.3 Test Samples and Referee Samples

3.3.1 From each of the selected containers, with the help of suitable sampling instrument, approximately equal quantity of material shall be taken out so as to make a composite sample of about 1 kg. This sample shall be thoroughly mixed and divided into three equal parts and transferred to clean and dry glass containers, sealed air tight and labelled with particulars as given in **3.1.5**. One of these composite samples shall be for the purchaser, another for the vendor and the third for the referee.

NOTE — In case the materials of various types are packed in the same container the material of each type shall be separated. The sample shall be prepared as given in **3.3.1** and test for conformity to requirements carried out separately for each type.

3.3.2 *Referee Sample* — Referee sample shall consist of the composite sample marked for this purpose and shall bear the seal of the purchaser and the vendor which shall be kept at a place as agreed to between the two. This shall be used in case of dispute between the purchaser and the vendor.

3.4 Number of Tests and Criteria for Conformity

3.4.1 All the requirements of the corresponding specification shall be tested on the composite sample (*see* **3.3.1**).

3.4.2 The lot shall be declared as conforming to the specification when the composite sample tested for various requirements shall satisfy the corresponding requirements of the specification.

4. PREPARATION OF SAMPLE

4.1 Mince as quickly as possible with a sharp-edged knife or grind in a dry pestle and mortar, 150 g of the sample on a clean porcelain slab. Mince thoroughly to secure a uniform sample. Store the minced sample immediately in an air-tight glass container and use this wherever the use of prepared sample is indicated.

5. DETERMINATION OF MOISTURE CONTENT (VACUUM DRYING METHOD)

5.1 Procedure — Weigh accurately about 5 g of the prepared sample (*see* **4.1**) in a tared aluminium flat dish with tight-fit cover having a diameter of about 75 mm and a height of about 25 mm. Distribute the material as evenly as practicable over the bottom of the dish by gentle sidewise movements. Place the dish in a vacuum oven, remove the cover of the dish and dry the material for two hours at 65 ± 1°C at a pressure not exceeding 50 mm of mercury. Run a current of dry air through the area during drying. Remove the dish from oven, cover, dry in a desiccator and weigh Redry for one hour and repeat the process till the difference between the two successive weighings is less than 2 mg. Allow the dish to cool to room temperature and weigh.

5.2 Calculations

5.2.1 Moisture, percent by mass $= \frac{100\,(M - M_1)}{M}$

where

M = mass in g of the prepared sample taken for the experiment, and

M_1 = mass in g of the material after drying.

NOTE 1 — Substances containing no fructose or other decomposable substance may be dried for 3 hours at 100°C at normal pressure.

NOTE 2 - In the case liquid or semi-liquid substances preliminary drying with quartz sand or pumice stone should be made on a steam bath.

Drying upon Pumice Stone — (Applicable to massecuites, molasses, and other liquid and semiliquid products) — Prepare pumice stone of 2 grades of fineness, one to pass through 1-mm sieve, other through 6-mm but not 1-mm sieve. Digest each for 8 hours with hydrochloric acid (1 : 4) on a steam bath. Wash acid-free and heat to 525°C. Make determination in flat metal dish of 60-mm diameter. Place 3 mm layer of the fine pumice stone on bottom of dish, then 6 to 10 mm layer of coarse pumice stone, dry and weigh. Dilute sample with weighed portion of water so that the diluted material contains about 20 to 30 percent of solid matter. Weigh into prepared dish an amount of diluted sample to yield about 1 g of dry matter. If this weighing cannot be made rapidly, use a weighing bottle provided with a cork through which a pipette passes. Dry at 70°C under pressure of not greater than

50 mm mercury (6·7 kPa) bleeding with dry air. Make trial weighings at 2 hours intervals towards end of drying period until change in mass is not more than 2 mg. Report loss in mass as water. Substances containing little or no fructose or other readily decomposable substance may be dried in an oven at 100°C.

Drying on Quartz Sand — (Applicable to massecuites, molasses, and other liquid and semiliquid products) — Digest pure quartz sand that passes through 425-micron but not through 250-micron sieve with hydrochloric acid, wash acid-free, dry and ignite. Preserve in a stoppered bottle. Place 20 to 30 g prepared sand and short stirring rod in dish about 55 mm diameter and 40 mm deep and fitted with a cover. Dry thoroughly, cover dish, cool in a desiccator and weigh immediately. Add enough diluted sample of known mass to yield about 1 g of dry matter and mix thoroughly with sand. Heat on a steam bath for 15 to 20 minutes stirring at 2 to 3 minutes intervals, or until mass becomes too stiff to manipulate readily. Dry at less than 70°C (preferably 60°C) under pressure not more than 50 mm mercury (6·7 kPa) bleeding with dry air. Make trial weighings at 2 hours intervals towards end of drying period (about 18 hours) until change in mass is not more than 2 mg. For materials containing no fructose or other readily decomposable substances dry for 8 to 10 hours at atmospheric pressure in an oven maintained at 100°C. Cool in a desiccator, and weigh, repeating heating and weighing until loss in 1 hour heating is not greater than 2 mg. Report loss in mass as water. (As dry sand, and dried sample absorbs appreciable moisture on standing over most desiccating agents, make all weighings as quickly as possible after cooling in a desiccator.)

6. DETERMINATION OF SULPHATED ASH

6.1 Reagent

6.1.1 *Sulphuric Acid* — 10 percent (*m/m*).

6.2 Procedure — Accurately weigh about 5 g of the prepared sample (*see* **4.1.1**) into a 9-cm diameter platinum basin. Add 5 ml of sulphuric acid to the material in the dish. Gently heat the dish on a hot plate until the material is well carbonized, and then increase the heat until the evolution of sulphuric acid fumes ceases. Ash the carbonized matter in a muffle furnace at 550 ± 25°C. Cool the ash and moisten it with 2-3 ml of sulphuric acid (*see* **6.1.1**). Heat strongly on a hot plate until sulphuric acid fumes cease to be evolved and finally ash in the muffle furnace at 550 ± 25°C for about 2 hours. Cool in a desiccator and weigh. Heat again in the muffle furnace for 30 minutes at 550 ± 25°C. Cool in a desiccator and weigh. Repeat the process of heating in the muffle furnace for 30 minutes, cooling and weighing till the difference between two successive weighings is less than 1 mg. Record the lowest mass.

6.3 Calculation

6.3.1 Ash, sulphated, percent by mass $= \frac{100\ M_1}{M_2}$

where

M_1 = mass in g of the ash, and

M_2 = mass in g of the prepared sample taken for the test.

7. DETERMINATION OF ACID INSOLUBLE ASH

7.1 Reagent

7.1.1 *Dilute Hydrochloric Acid* — Approximately 5 N (prepared from concentrated hydrochloric acid).

7.2 Procedure — Weigh accurately about 5 g of the prepared sample (*see* **4.1**) in a tared, clean and dry platinum basin of 100 ml capacity. Carbonize the material in the dish with the flame of a burner. Complete the ignition by keeping in a muffle furnace at 550 ± 25°C until grey ash results. Cool in a desiccator. To the ash, add 25 ml of the dilute hydrochloric acid, cover with a watch glass and heat on a small flame of a burner to near boiling. Allow to cool and filter the contents of dish through Whatman filter paper No. 42 or its equivalent. Wash the filter with hot water until the washings are free from chlorides. Return the filter and the residue to the dish. Keep it in an air-oven maintained at 105 ± 2°C for about 3 hours. Ignite in the muffle furnace at 550 ± 25°C for one hour. Cool the dish in a desiccator and weigh. Heat again for 30 minutes in the muffle furnace, cool and weigh. Repeat this process of heating for 30 minutes, cooling and weighing till the difference between two successive weighings is less than one milligram. Note the lowest mass.

7.3 Calculation

7.3.1 Acid insoluble ash, percent by mass $\frac{100\ M_1}{M_2}$

where

M_1 = mass in g of the acid insoluble ash, and

M_2 = mass in g of the prepared sample taken for the test.

8. DETERMINATION OF REDUCING SUGARS

8.1 Reagents

8.1.1 *Stock Solution of Dextrose* — Weigh accurately 10 g of anhydrous dextrose into a 1-litre graduated flask and dissolve it in water. Add to this solution 2·5 g of benzoic acid, shake to dissolve the benzoic acid and make up the volume to the mark with water. (This solution shall not be used after 48 hours).

8.1.2 *Standard Dextrose Solution* — Dilute a known aliquot of the stock solution of dextrose (*see* **8.1.1**) with water to such a concentration that more than 15 ml but less than 50 ml of it will be required to reduce all the copper in the Fehling's solution taken for titration. Note the concentration of anhydrous dextrose in this solution as milligrams per 100 ml (*see* Note), prepare this solution fresh every day.

NOTE — When 10 ml (*see* **8.3.1**) of Fehling's solution are taken for titration, a standard dextrose solution containing 0·11 to 0·30 percent (*m/v*) of anhydrous dextrose is convenient for use.

8.1.3 *Methylene Blue Indicator Solution* — Dissolve 0·2 g of methylene blue in water and dilute to 100 ml.

8.1.4 *Petroleum Ether* — Re-distilled below 60°C.

8.1.5 *Fehling's Solution* (*Soxhlet Modification*) — Prepared by mixing immediately before use, equal volumes of solution A and solution B.

8.1.5.1 *Solution A* — Dissolve 34·639 g of copper sulphate ($CuSO_4$ $5H_2O$) in water, add 0·5 ml of concentrated sulphuric acid of sp gr 1·84 and dilute to 500 ml in a graduated flask. Filter the solution through prepared asbestos.

8.1.5.2 *Solution B* — Dissolve 173 g of potassium sodium tartrate ($KNaC_4H_4O_6$, $4H_2O$) (Rochelle salt) and 50 g of sodium hydroxide, analytical reagent in water, dilute to 500 ml in a graduated flask and allow the solution to stand for two days. Filter the solution through prepared asbestos or filter paper (Whatman No. 4).

8.1.5.3 *Standardization of Fehling's solution* — Pour standard dextrose solution (*see* **8.1.2**) into a 50-ml burette (*see* Note 3 under **8.2.3**). Find the titre (that is the volume of standard dextrose solution required to reduce all the copper in 10 ml of Fehling's solution) corresponding to the concentration of standard dextrose solution from Table 2. (If, for example, the standard dextrose solution contains 167·0 mg of anhydrous dextrose per 100 ml, the corresponding titre would be 30 ml). Pipette 10 ml (*see* **8.3.1.1**) of Fehling's solution into a 300-ml conical flask and run in from the burette almost the whole of the standard dextrose solution required to effect reduction of all the copper, so that not more than one millilitre will be required later to complete the titration. Heat the flask containing the mixture over a wire gauze. Gently boil the contents of the flask for 2 minutes. At the end of 2 minutes of boiling, add without interrupting boiling, one millilitre of methylene blue indicator solution. While the contents of the flask continue to boil, begin to add standard dextrose solution (one or two drops at a time) from the burette till the blue colour of the indicator just disappears. [The titration should be completed within one minute so that the contents of the flask boil together for 3 minutes without interruption (*see* Note 2 under **8.2.3**)]. Note the titre (that is the total volume in

millilitres of standard dextrose solution used for the reduction of all the copper in 10 ml of Fehling's solution). Multiply the titre (obtained by direct titration) by the number of milligrams of anhydrous dextrose in one millilitre of the standard dextrose solution to obtain the dextrose factor. Compare this factor with the dextrose factor given in Table 2 and determine correction, if any, to be applied to the dextrose factor derived from Table 2.

Example:

Concentration in mg/100 ml of anhydrous dextrose in standard dextrose solution	= 167·0
Titre in ml obtained by direct titration	= 30·1
Dextrose factor for 30·1 ml of standard dextrose solution	= Titre in ml × number of mg of anhydrous dextrose in one millilitre of standard dextrose solution
	= 30·1 × 1·670
	= 50·267 0
Dextrose factor for 30·1 ml of standard dextrose solution from Table 2 (calculated by interpolation)	= 50·11
Correction to be applied to the dextrose factor derived from Table 2	= 50·267 0 — 50·11
	= + 0·157 0

8.1.6 *Zinc Acetate Solution* — Dissolve 21 9 g of zinc acetate [$Zn (C_2H_3O_2)_2, 2H_2O$] and 3 ml of glacial acetic acid in water. Dilute to 100 ml.

8.1.7 *Potassium Ferrocyanide Solution* — A 10·6 percent aqueous solution.

8.2 Procedure

8.2.1 *Preparation of Solution* — Weigh accurately about 5 g of sample in a beaker and transfer into a 200-ml volumetric flask quantitatively with the aid of warm water Dilute to about 150 ml. In case the solution is not sufficiently clear, ad l with gently mixing 5 ml zinc acetate solution, and mix, followed by 5 ml of potassium ferrocyanide solution in the same manner. Make the volume up to the 200-ml mark Filter through a Whatman filter paper No. 40 or its equivalent. Collect the filtrate for titration

TABLE 2 DEXTROSE FACTORS FOR 10 ml OF FEHLING'S SOLUTION

(*Clauses* 8.1.5.3 *and* 8.3.1)

TITRE	DEXTROSE FACTOR*	DEXTROSE CONTENT PER 100 ml OF SOLUTION
(1)	(2)	(3)
ml		mg
15	49·1	327
16	49·2	307
17	49·3	289
18	49·3	274
19	49·4	260
20	49·5	247·4
21	49·5	235·8
22	49·6	225·5
23	49·7	216·1
24	49·8	207·4
25	49·8	199·3
26	49·9	191·8
27	49·9	184·9
28	50·0	178·5
29	50·0	172·5
30	50·1	167·0
31	50·2	161·8
32	50·2	156·9
33	50·3	152·4
34	50·3	148·0
35	50·4	148·9
36	50·4	140·0
37	50·5	136·4
38	50·5	132·9
39	50·6	129·6
40	50·6	126·5
41	50·7	123·6
42	50·7	120·8
43	50·8	118·1
44	50·8	115·5
45	50·9	113·0
46	50·9	110·6
47	51·0	108·4
48	51·0	106·2
49	51·0	104·1
50	51·1	102·2

*Milligams of anhydrous dextrose corresponding to 10 ml of Fehling's solution.

8.2.2 *Incremental Method of Titration* — Pour the prepared solution (*see* **8.2.1**) into a 50-ml burette (*see* Note 3 under **8.2.3**). Pipette 10 ml of Fehling's solution into a 300-ml conical flask and run in from the burette 15 ml of the prepared solution Without further dilution, heat the contents of the flask over a wire gauze, and boil. (After the liquid has been boiling for about 15 seconds it will be possible to judge if almost all the copper is reduced by the bright red colour imparted to the boiling liquid by the suspended cuprous oxide). When it is judged that nearly all the copper is reduced, add one millilitre of methylene blue indicator solution (*see* Note 1). Continue boiling the contents of the flask for one to two minutes from the commencement of ebullition, and then add the prepared solution in small quantities (one millilitre or less at a time) allowing the liquid to boil for about 10 seconds between successive additions, till the blue colour of the indicator just disappears (*see* Note 2 under **8.2.3**) In case there still appears to be much unreduced copper after the mixture of Fehling's solution with 15 ml of the prepared solution has been boiling for 15 seconds, add the prepared solution from the burette in larger increments (more than one millilitre at a time according to judgement) and allow the mixture to boil for 15 seconds after each addition. Repeat the addition of the prepared solution at intervals of 15 seconds until it is considered unsafe to add a large increment of the prepared solution. At this stage, continue the boiling for an additional one to two minutes, add one millilitre of methylene blue indicator solution and complete the titration by adding the prepared solution in small quantities (less than one millilitre at a time) (*see also* Note 2).

NOTE 1 — It is advisable not to add the indicator until the end point has been nearly reached because the indicator retains its full colour until the end point is almost reached and thus gives no warning to the operator to go slowly.

NOTE 2 — When the operator has had a fair amount of experience with the method, a sufficiently accurate result may often be obtained by a single estimation by the incremental method of titration For the utmost degree of accuracy of which the method is capable, a second titration should be carried out by the standard method of titration (*see* **8.2.3**).

8.2.3 *Standard Method of Titration* — Pipette 10 ml of Fehling's solution into a 300-ml conical flask and run in from the burette almost the whole of the prepared solution required to effect reduction of all the copper (determined under **8.2.2**), so that, if possible, not more than one millilitre will be required later to complete the titration. Gently boil the contents of the flask for 2 minutes. At the end of 2 minutes of boiling, add without interrupting boiling, one millilitre of methylene blue indicator solution. While the contents of the flask continue to boil, begin to add the prepared solution (one or two drops at a time) from the burette till the blue colour of the indicator just disappears (*see* Note 1). [The titration should be completed within one minute so that the contents of the flask boil altogether for 3 minutes without interruption (*see* Note 2)]

In case of doubt, the flame may be removed from the wire gauze for one or two seconds and the flask held against a sheet of white paper. (A holder of paper, suitably fixed around the neck of the flask is very convenient for this purpose as it can be left round the neck of the flask without risk of overbalancing it.) The top edge of the liquid would appear bluish if the indicator is not completely decolourized. It is inadvisable to interrupt the boiling for more than a few seconds as the indicator undergoes back oxidation rather rapidly when air is allowed free access into the flask, but there is no danger of this as long as a continuous stream of steam is issuing from the mouth of the flask.

NOTE 1 — The indicator is so sensitive that it is possible to determine the end point within one drop of the prepared solution in many cases. The complete decolouration of the methylene blue is usually indicated by the whole reaction liquid, in which the cuprous oxide is continuously churned up, becoming bright red or orange in colour.

NOTE 2 — It should be observed that with both incremental and standard methods of titration, the flask containing the reaction mixture is left on the wire gauze over the flame throughout the titration, except when it may be removed for a few seconds to ascertain if the end point is reached.

NOTE 3 — In adding sugar solution to the reaction mixture, the burette may be held in hand over the flask. The burette may be fitted with a small outlet tube bent twice at right angles, so that the body of the burette can be kept out of the steam while adding sugar solution. Burettes with glass taps are unsuitable for this work as the taps become heated by the steam and are liable to jam.

8.3 Calculation

8.3.1 Refer to Table 2 for the dextrose factor corresponding to the titre (determined as given under **8.2.3**) and apply the correction previously determined under **8.1.5.3**. Calculate the dextrose content of the prepared solution (*see* **8.2.1**) as follows:

$$\text{Milligrams of anhydrous dextrose present in one millilitre of the prepared solution } (M) = \frac{\text{Dextrose factor}}{\text{Titre}}$$

8.3.1.1 Instead of using 10 ml of Fehling's solution, a 25-ml portion may also be substituted throughout the procedure (including standardization of Fehling's solution under **8.1.5.3**). In this case, the standard dextrose solution, used in standardizing the Fehling's solution and the prepared solution of the material (*see* **8.2.1**) shall contain 0·25 to 0·75 percent (*m/v*) of anhydrous dextrose, and Table 3 shall be used for all calculation.

8.3.2 Reducing sugars, percent by mass $= \frac{m}{M} \times 10$

where

m = milligrams of anhydrous dextrose in 1 ml of the solution of the material (*see* **8.3.1**), and

M = mass in g of the prepared sample used for making 100 ml of solution (*see* **8.2.1**).

TABLE 3 DEXTROSE FACTORS FOR 25 ml OF FEHLING'S SOLUTION

(*Clause* 8 3 1.1)

TITRE	DEXTROSE FACTOR*	DEXTROSE CONTENT PER 100 ml OF SOLUTION
ml		mg
(1)	(2)	(3)
15	120·2	801
16	120·2	751
17	120·2	707
18	120·2	668
19	120·3	638
20	120 3	601 5
21	120·3	572·9
22	120·3	547·3
23	120·4	523 6
24	120·5	501 9
25	120·5	482 0
26	120·6	463 7
27	120·6	446·8
28	120 7	431 1
29	120·7	416·4
30	120 8	402 7
31	120·8	389 7
32	120 8	377 6
33	120·9	366 3
34	120·9	355·6
35	121·0	345·6
36	121·0	336·3
37	121·1	327 4
38	121·2	318 8
39	121·2	310 7
40	121·2	303·1
41	121·3	295 9
42	121 4	289 0
43	121·4	282 4
44	121·5	276·1
45	121 5	270 1
46	121 6	264 3
47	121 6	258 8
48	121·7	253 5
49	121 7	248 4
50	121 8	243 6

NOTE — Tables 2 and 3 show, for the standard method of titration, the values correponding to integral millilitres of the sugar solution, intermediate values being obtained by interpolation.

*Milligrams of anhydrous dextrose corresponding to 25 ml of Fehling's solution.

9. DETERMINATION OF SUCROSE

9.1 Reagents

9.1.1 *Concentrated Hydrochloric Acid* – sp gr 1·16, analytical reagent grade.

9.1.2 *Fehling's Solution (Soxhlet Modification)* — Prepared by mixing immediately before use, equal volumes of Solution A and Solution B.

9.1.2.1 *Solution A* — Dissolve 34·639 g of copper sulphate ($CuSO_4.5H_2O$) in water, add 0·5 ml of concentrated sulphuric acid of sp gr 1·84 and dilute to 500 ml in a graduated flask. Filter the solution through prepared asbestos.

9.1.2.2 *Solution B* — Dissolve 173 g of potassium sodium tartrate ($KNaC_4H_4O_6$, $4H_2O$) Rochelle salt and 50 g of sodium hydroxide, analytical reagent, in water. Dilute to 500 ml in a graduated flask and allow the solution to stand for two days. Filter this solution through prepared asbestos.

9.2 Procedure — Take 10 ml of the prepared solution (*see* **8.2.1**) in a conical flask and add 1·5 ml of the concentrated hydrochloric acid and about 10 ml of water. Heat the flask at 60 to 70°C for 10 minutes in a water-bath Cool immediately and neutralize with 30 percent sodium hydroxide (*m/v*) and transfer quantitatively the neutralized inverted solution to a graduated flask and make up the volume to 100 ml.

Determine the reducing sugars in the inverted solution as described in **8.**

9.3 Calculation

9.3.1 Sucrose, percent by mass $= \frac{(O - RM)\,0{\cdot}95}{M}$

where

O = the value in col 3 of Table 3 corresponding to the titre;

R = reducing sugars, percent by mass (*see* **8**); and

M = mass in g of the original material taken for the test (*see* **8.2.1**).

10. DETERMINATION OF FAT

10.0 Two methods for the determination of fat are given. Any of these may be used. If the confectionery is known to contain milk, method No. 2 (Roese-Gottlieb Method) wherein ammonia is used to dissolve the milk protein before fat extraction may be used.

10.1 Simple Extraction Method

10.1.1 *Apparatus*

10.1.1.1 Mojonnier fat extraction tube or any other similar apparatus.

10.1.1.2 *Flasks*

10.1.2 *Reagents*

10.1.2.1 *Diethyl ether* — peroxide free.

10.1.2.2 *Petroleum ether* — boiling range 40 to 60°C.

10.1.3 *Procedure* — Dissolve 10 g sample in 10 ml of warm water and introduce into Mojonnier fat extraction tube or similar apparatus. Add 25 ml peroxide free diethyl ether. Cork the tube and shake vigorously for 1 minute. Add 25 ml of petroleum ether and shake vigorously for 30 seconds. Let it stand for 30 minutes or until separation is complete. Draw off the fat solution into a suitable flask (previously dried at 100°C, cooled and weighed). Repeat the extraction and subsequent operations twice more. Evaporate the ether and dry the fats for 1 hour at 100°C. Cool and weigh.

10.1.4 *Calculations*

10.1.4.1 Fat, percent by mass (on dry basis) $= \frac{M_1}{M_2 (100 - M)}$

where

M_1 = mass in g of extracted fat,

M_2 = mass in g of the prepared sample taken, and

M = percentage of moisture in the material.

10.2 Roese-Gottlieb Method

10.2.1 *Apparatus*

10.2.1.1 *Mojonnier fat extraction tube or similar apparatus*

10.2.2 *Reagents*

10.2.2.1 *Concentrated ammonia solution* — sp gr 0·88.

10.2.2.2 *Ethyl alcohol* — 95 to 96 percent (*v/v*).

10.2.2.3 *Diethyl ether* — sp gr 0·720 (peroxide free).

10.2.2.4 *Petroleum ether* — boiling range 40 to 60°C, recently distilled

10.2.3 *Method*

10.2.3.1 Introduce 4 g sample or amount of uniform solvent equivalent to this weight of dry substance into a Mojonnier fat extraction tube or similar apparatus. Dilute to 10 ml with water. Add 1·2 ml ammonia solution and mix thoroughly. Add 10 ml alcohol and mix; then add 25 ml ether and shake vigorously for about 30 seconds and finally add 25 ml petroleum ether and shake again for about 30 seconds. Let stand for 20 minutes or until separation of liquids is complete.

10.2.3.2 Draw off as much as possible of ether-fat solution (usually 0·5 to 0·8 ml is left) into a weighed flask through a small rapid filter. Weigh flask with a similar one as counterpoise. Again extract liquid remaining in tube, this time with 15 ml each of ether and petroleum ether; shake vigorously for about 30 seconds with each solvent and let settle. Proceed as above, washing mouth of tube and filter with a few millilitres of mixture of equal parts of the two solvents (previously mixed and freed from deposited water).

10.2.3.3 For greater degree of accuracy, repeat extraction. If previously solvent-fat solutions have been drawn off closely, third extraction usually yields approximately up to 1 mg fat or about 0 02 percent with 4 g sample. Slowly evaporate solvent on steam bath and then dry fat in an oven maintained at 100°C to constant mass. Test purity of fat by dissolving in a little petroleum ether. If residue remains, wash out fat completely with petroleum ether, dry the residue, weigh and calculate the mass of the fat.

11. DETERMINATION OF PROTEIN

11.1 Apparatus

11.1.1 A recommended apparatus, as assembled, is shown in Fig. 1.

11.1.1.1 *Description* — The assembly consists of a round bottom flask *A* of 1 000 ml capacity fitted with a rubber stopper through which passes one end of the connecting bulb tube *B*. The other end of the bulb *B* is connected to the condenser *C* which is attached, by means of a rubber tube, to a dip tube *D* which dips into a known quantity of standard sulphuric acid contained in a beaker *E* of 250 ml capacity.

11.1.2 *Kjeldahl Flask* — Capacity 500 ml.

11.2 Reagents

11.2.1 *Anhydrous Sodium Sulphate*

11.2.2 *Copper Sulphate*

11.2.3 *Concentrated Sulphuric Acid* — sp gr 1·84

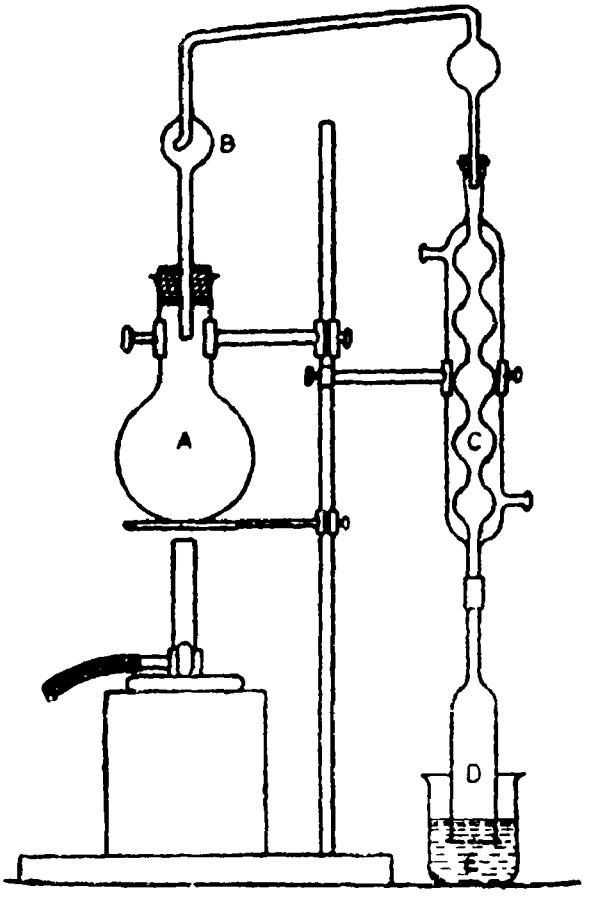


FIG. 1 DISTILLATION ASSEMBLY

11.2.4 *Sodium Hydroxide Solution* — Dissolve about 225 g of sodium ydroxide in 500 ml of water.

11.2.5 *Standard Sulphuric Acid* — 0·1 N.

11.2.6 *Methyl Red Indicator Solution* — Dissolve one gram of methyl red ı 200 ml of rectified spirit (95 percent *v/v*)

11.2.7 *Standard Sodium Hydroxide Solution* — 0·1 N.

11.3 Procedure

11.3.1 Transfer carefully about one to two grams of the material accurately weighed, to the Kjeldahl flask, taking precaution to see that particles of the material do not stick to the neck of the flask. Add about 10 g of anhydrous sodium sulphate, about 0·2 to 0·3 g of copper sulphate and 20 ml of concentrated sulphuric acid. Place the flask in an inclined position. Heat below the boiling point of the acid until frothing ceases. Increase heat until the acid boils vigorously and digest for 30 minutes after the mixture becomes clear and pale green or colourless. Cool the contents of the flask. Transfer quantitatively to the round bottom flask with water, the total quantity of water used being about 200 ml Add with shaking a few pieces of pumice stone to prevent bumping Add about 50 ml of the sodium hydroxide solution (which is sufficient to make the solution alkaline) carefully through the side of the flask so that it does not mix at once with the acid solution but forms a layer below the acid layer. Assemble the apparatus as shown in Fig. 1 taking care that the dip tube extends below the surface of the standard sulphuric acid contained in the beaker. Mix the contents of the flask by shaking and distil until all ammonia has passed over into the standard sulphuric acid. Shut off the burner and immediately detach the flask from the condenser. Rinse the condenser thoroughly with water into the beaker. Wash the dip tube carefully so that all traces of the condensate are transferred to the beaker When all the washings have been drained into the beaker, add two or three drops of methyl red indicator solution and titrate with the standard sodium hydroxide solution.

11.3.2 Carry out a blank determination using all reagents in the same quantities but without the material to be tested

11.4 Calculation

11.4.1 Total protein ($N \quad 6{\cdot}25$) percent by mass $= \dfrac{8{\cdot}75\,(B - A)\,N}{M}$

where

B = volume in ml of the standard sodium hydroxide solution used to neutralize the acid in the blank determination,

A = volume in ml of the standard sodium hydroxide solution used to neutralize the excess of acid in the test with the material,

N = normality of the standard sodium hydroxide solution, and

M = mass in g of the material taken for the test.

12. DETERMINATION OF SULPHUR DIOXIDE

12.1 Apparatus

12.1.0 The apparatus, as assembled, is shown in Fig. 2.

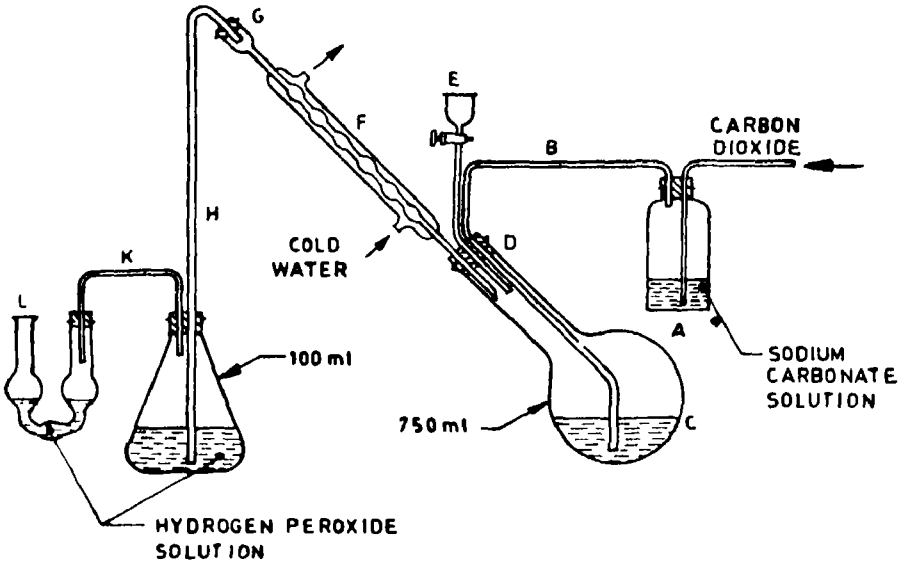


FIG. 2 ASSEMBLY OF APPARATUS FOR THE DETERMINATION OF SULPHUR DIOXIDE

12.1.1 The apparatus consists of the round bottom flask *C* of capacity 750 ml fitted with the three-hole stopper *D*. The rubber stopper *D* is fitted with the delivery tube *B*, the dropping funnel *E*, and the sloping, water-cooled, reflux condenser *F*, the lower end of which is cut off at an angle. The free end of delivery tube *B* is connected to the wash bottle *A* containing sodium carbonate solution. The upper end of the reflux condenser *F* is connected to the delivery tube *H* by the rubber stopper *G*. The free end of the delivery tube *H* nearly reaches the bottom of the 100 ml Erlenmeyer flask *J* containing 25 ml of hydrogen peroxide solution. The Erlenmeyer flask *J* is provided with a two-hole rubber stopper; through one hole passes the delivery tube *H* and through the other tube *K*. The free end of the tube *K* is connected to the Peligot tube *L* containing 5 ml of hydrogen peroxide solution.

12.2 Reagent

12.2.1 *Sodium Carbonate Solution* — 10 percent (*m/v*), aqueous.

12.2.2 *Bromophenol Blue Indicator Solution* — Dissolve 0·1 g of bromophenol blue in 3·0 ml of 0·05 N sodium hydroxide solution and 5 ml of ethyl alcohol (90 percent by volume) by gently warming. Make up the volume of the solution with ethyl alcohol (20 percent *v/v*) to 250 ml in a volumetric flask.

12.2.3 *Hydrogen Peroxide Solution* — Dilute a 30 percent (*m/v*) hydrogen peroxide solution with about twice its volume of water and neutralize the free sulphuric acid that may be present in the hydrogen peroxide solution with barium hydroxide solution, using bromophenol blue indicator solution. Allow the precipitate of barium sulphate to settle, and filter. Determine the concentration of hydrogen peroxide in the filtrate by titrating with standard potassium permanganate solution. Dilute the filtrate with cold water so as to obtain a 3 percent (*m/v*) *solution of hydrogen peroxide.*

12.2.4 *Concentrated Hydrochloric Acid* — sp gr 1·16.

12.2.5 *Carbon Dioxide Gas* — from a cylinder.

12.2.6 *Standard Sodium Hydroxide Solution* — Approximately 0·1 N, standardized at the time of the experiment using bromophenol blue indicator solution.

12.3 Procedure

12.3.1 Assemble the apparatus as shown in Fig. 2. Introduce into the flask *C*, 300 ml of water and 20 ml of concentrated hydrochloric acid through the dropping funnel *E*. Run a steady current of cold water through the condenser *F*. Boil the mixture contained in the flask *G* for a short time to expel air from the system in current of carbon dioxide gas previously passed through the wash bottle *A*. Weigh accurately about 1·00 g of the material and mix with the minimum quantity of water so as to make the diluted material easily flow down to the dropping funnel. Introduce the diluted material into the flask *C* through the dropping funnel *E*. Wash the dropping funnel with a small quantity of water and run the washing into the flask *C*. Again boil the mixture contained in the flask *C* in a slow current of carbon dioxide gas (passed previously through the wash bottle *A*) for one hour. Just before the end of the distillation, stop the flow of water in the condenser (This causes the condenser to become hot and drives over residual traces of sulphur dioxide retained in the condenser). When the delivery tube *H*, just above the Erlenmeyer flask *J*, becomes hot to touch, remove the stopper *J* immediately. Wash the delivery tube *H* and the contents of the Peligot tube *L* with water into the Erlenmeyer flask. Cool the contents of the Erlenmeyer flask to room temperature, add a few drops of bromophenol blue indicator solution and titrate with standard sodium hydroxide solution (Bromophenol blue is unaffected by carbon dioxide and gives a distinct change of colour in cold hydrogen peroxide solution).

12.3.2 Carry out a blank determination using 20 ml of concentrated hydrochloric acid diluted with 300 ml of water.

12.4 Calculation

12.4.1 Sulphur dioxide, mg/kg $= \frac{32\,030\,(V - v)\,N}{M}$

where

V = volume in ml of standard sodium hydroxide solution required for the test with the material;

v = volume in ml of standard sodium hydroxide solution required for the blank determination;

N = normality of standard sodium hydroxide solution; and

M = mass in g of the material taken for the test.

13. DETERMINATION OF ARSENIC

13.0 *Two methods for the determination of arsenic have been prescribed,* namely, the visual comparison method and the silver diethyldithiocarbamate method. Any of the two methods may be used, however for referee purposes the silver diethyldithiocarbamate method shall be used. This method is however applicable for quantities of arsenic (as As) in the range 1 to 20 μg.

13.1 Visual Comparison Method

13.1.1 *Apparatus*

13.1.1.1 *Distillation apparatus* — Assembled as shown in Fig. 3.

13.1.1.2 *Apparatus for the determination of arsenic* — Assembled as shown in Fig. 4. The apparatus consists of the following parts:

a) *Wide-mouth bottle* — Capacity 120 ml.

b) *Glass tube* — Made from ordinary glass tubing and having a total length of 200 mm. It should have an internal diameter of exactly 6·5 mm and an external diameter of about 8 mm. It is drawn out at one end to a diameter of about one millimetre and a hole not less than 2 mm in diameter is blown in the side of the tube, near the constricted part The upper end of the tube is cut off square and is either rounded off slightly or ground smooth.

c) *Rubber bungs* — Three. One fits exactly into the mouth of the wide-mouth bottle and has a hole bored centrally to take the tube from its constricted end. Each of the other two rubber bungs (about 25 × 25 mm) has a hole, exactly 6 5 mm in diameter, bored centrally and are fitted with a rubber band or spring clip for holding them tightly together.

d) *Preparation of the glass tube* — Moisten a small quantity of cotton wool with lead acetate solution and then dry it in a dust free atmosphere. Lightly pack the glass tube with this cotton wool, so that the upper surface of the cotton wool is not less than 25 mm below the top of the tube. Insert the upper end of the tube into the narrow end of one of the pair of rubber bungs either to a depth of about 10 mm (when the tube has rounded off end) or to such an extent that the ground end of the tube is flush with larger end of the bung. Place a piece of mercuric chloride paper flat on the top of the bung Place the other bung over this with its larger end in contact with the piece of mercuric chloride paper. Fasten the two bungs by means of the rubber band or the spring clip in such a manner that the borings of the two bungs (or the upper bung and the glass tube) meet to form a true tube of 6·5 mm diameter interrupted by a diapharagm of mercuric chloride paper.

Instead of this method of attaching the mercuric chloride paper any other method may be used provided that (a) the whole of the evolved gas passes through the paper, (b) the portion of the paper in contact with the gas is a circle of 6·5 mm diameter, and (c) the paper is protected from sunlight during the test.

13.1.2 *Reagents*

13.1.2.1 *Dilute nitric acid* — 30 percent.

13.1.2.2 *Concentrated sulphuric acid* — sp gr 1 84

13.1.2.3 *Concentrated nitric acid* — sp gr 1·42.

13.1.2.4 *Chloride-hydrazine-bromide mixture* — Mix 5 g of sodium chloride, 0·5 g of hydrazine sulphate and 0·02 g of potassium bromide followed rapidly by 10 ml of concentrated hydrochloric acid and store in a tightly stoppered bottle.

13.1.2.5 *Lead acetate solution* — 10·0 percent (*m/v*) in distilled water recently boiled.

13.1.2.6 *Mercuric chloride paper* — Smooth white filter paper, not less than 25 mm in width, soaked in a saturated solution of mercuric chloride in water; pressed to remove superfluous solution, and dried at 60°C in the dark. The grade of the filter paper shall be such that the mass in g/m^2 shall be between 65 and 120; the thickness in mm of 400 papers shall be approximately equal, numerically, to the mass in g/m^2. Same quality of paper shall be used for the control and experiment. Mercuric chloride paper should be stored in a stoppered bottle in the dark. Paper which has been exposed to sunlight or to the vapour of ammonia should not be used as they give a lighter coloured stain or no stain at all when employed in the quantitative test for arsenic

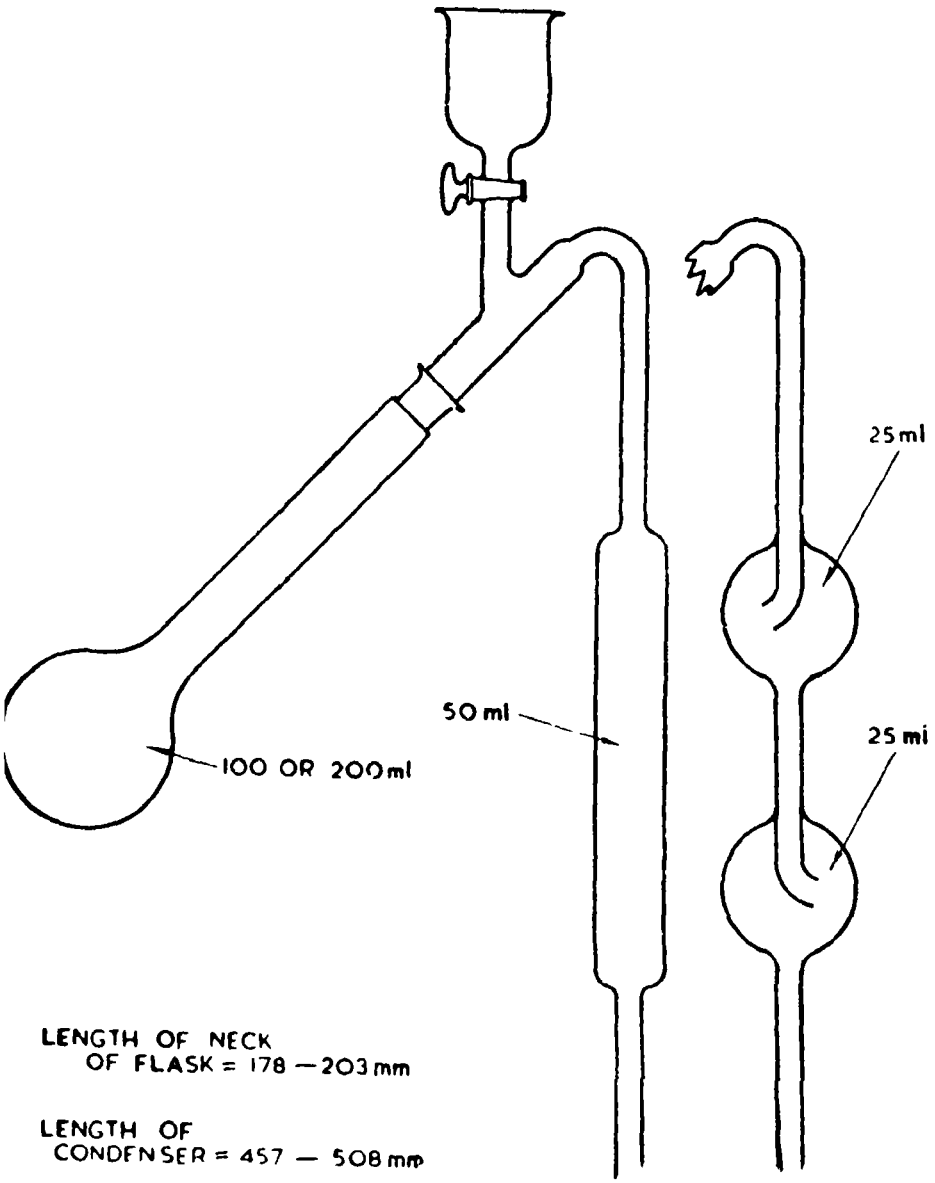


FIG. 3 DISTILLATION APPARATUS

13.1.2.7 *Concentrated hydrochloric acid* — sp gr 1·16.

13.1.2.8 *Stannous chloride solution* — Dilute 60 ml of concentrated hydrochloric acid with 20 ml of water, add to it 20 g of tin, heat gently until gas ceases to be evolved, and add sufficient water to produce 100 ml, allowing the undissolved tin to remain in the solution. Decant the clear solution, add an equal volume of concentrated hydrochloric acid, boil down to the original volume and filter through a fine-grain filter paper.

13.1.2.9 *Stannated hydrochloric acid* — Mix together one millilitre of stannous chloride solution and 100 ml of concentrated hydrochloric acid.

13.1.2.10 *Potassium iodide* — Crystals or in the form of powder.

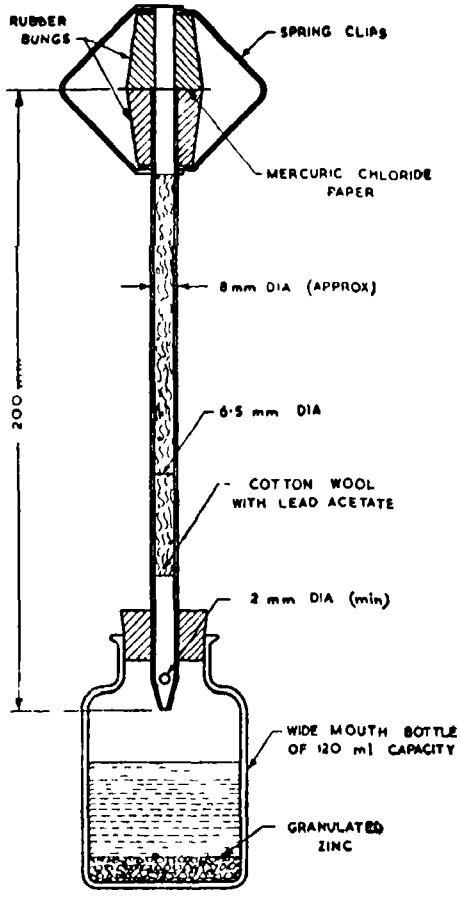


FIG. 4 ASSEMBLY OF APPARATUS FOR THE DETERMINATION OF ARSENIC

13.1.2.11 *Zinc* — Granulated and complying with the following test:

Take 50 ml of water, 10 ml of stannated hydrochloric acid and 0·1 ml of dilute solution of arsenic (*see* **13.1.2.13**) in the wide-mouth bottle. Add one gram of potassium iodide and 10 g of zinc. Quickly place the prepared glass tube (*see* **13.1.1.2**) in position. Allow the reaction to continue for one hour. A faint but distinct yellow stain shall be produced on the mercuric chloride paper.

13.1.2.12 *Strong solution of arsenic* — Dissolve 0·132 g of arsenic trioxide in 50 ml of concentrated hydrochloric acid and add sufficient water to produce 100 ml.

13.1.2.13 *Dilute solution of arsenic* — Freshly prepared. Dilute one millilitre of strong solution of arsenic with water sufficient to produce 100 ml. This solution contains 0·01 mg of arsenic per millilitre.

13.1.3 *Procedure*

13.1.3.1 *Preparation of the solution* — Weigh 4·5 to 5·5 g of the prepared sample (*see* **4.1**) to an accuracy of 0·01 g and place with 10 ml of dilute nitric acid in a 100 or 200 ml resistance glass or silica Kjeldahl flask, and heat the mixture until any initial vigorous reaction subsides and ceases. Cool and add gradually 10 ml of concentrated sulphuric acid at such a rate as to present excessive frothing or heating (10 minutes are usually required) and continue the heating. Add to the hot solution 5 ml of concentrated nitric acid in small portions, and boil until colourless. If necessary, add concentrated nitric acid in further small portions at a time Note for the purpose of the blank determination the total amount of concentrated nitric acid added. (The digestion usually takes about 30 minutes). Cool, dilute with 50 ml of water and transfer to the flask of the distillation apparatus. Boil the solution without inserting the condensing arm till the bulk is reduced to about 10 ml or until white fumes appear; cool, dilute and again boil down to 10 ml; cool and add 7·0 ml of water. Cool well the liquid and add 5 g of the chloride-hydrazine-bromide mixture. Fit the condenser quickly and distil the liquid into a mixture of 10 ml of water and 2 ml of concentrated nitric acid. Then evaporate the distillate to dryness on the water-bath and evaporate the residue twice to dryness with 5 ml of water to remove nitric acid. Dissolve the final residue by warming in 3 ml of concentrated sulphuric acid, cool and dilute with water.

13.1.3.2 Transfer the whole of the prepared solution to the wide-mouth bottle, add 15 ml of stannated hydrochloric acid and one gram of potassium iodide. Then add 10 g of zinc. Quickly place the prepared glass tube [*see* **13.1.1.2** (b)] in position. Allow the reaction to continue for 40 minutes. Remove the piece of mercuric chloride paper at the end of this period. If arsenic is present in the material, compare the yellow

stain produced on the mercuric chloride paper, by daylight with the standard stains prepared as described under **13.1.3.4**:

a) If the stain in this test exceeds that produced by 0 02 mg of arsenic (that is, 2 2 ml of solution), make the solution to a known bulk with dilute sulphuric acid (1 : 8) and take an aliquot to produce a stain suitable for matching.

b) The reaction may be accelerated by placing the apparatus on a warm surface, care being taken that the mercuric chloride paper remains quite dry throughout the test. The most suitable temperature for carrying out the test is generally about 40°C, but because the rate of evolution of the gas varies somewhat with different batches of zinc, the temperature may be adjusted to obtain a regular, but not too violent, evolution of gas. The tube should be washed with concentrated hydrochloric acid, rinsed with water, and dried between successive tests.

13.1.3.3 *Comparison of stains* — The comparison of the stains is made with freshly prepared standard stain immediately at the completion of the test.

13.1.3.4 *Preparation of standard stains* — Prepare a series of solutions by mixing together different quantities (ranging up to 2 0 ml) of dilute solution of arsenic with 50 ml of water and 10 ml of stannated hydrochloric acid. Treat each solution in the series separately as described under **13.1.3.2** to prepare the series of standard stains.

13.1.3.5 Make sure that no solid material comes in contact with the ground in portion of the bottle.

13.1.3.6 A blank determination shall be carried out under the same conditions, on the same reagents and by the same person but without using the material. The blank should not produce any visible stain on the mercuric chloride paper

13.1.4 *Calculation*

13.1.4.0 Express the arsenic content of the material as milligrams of arsenic (As) per kilogram of the material.

13.1.4.1 Calculate the arsenic content by using the formula given either in (a) or (b) as appropriate:

a) Arsenic (As), mg/kg $= \frac{10\,A}{M}$

b) Arsenic (As), mg/kg $= \frac{10\,A\,V}{v\,M}$

where

A = volume in ml of dilute solution of arsenic used to prepare the standard stain that matches with the stain prepared from the material (*see* **13.1.3.4**);

M = mass in g of the ' prepared sample' used in the preparation of the solution of the material (*see* **13.1.3.1**);

V = total volume in ml of the solution made from the residue of the distillate with dilute sulphuric acid (1 : 8) [*see* **13.1.3.2** (a)]; and

v = volume in ml of the solution of the residue of the distillate used in the preparation of the stain [*see* **13.1.3.2** (a)].

13.2 Silver Diethyldithiocarbamate Method

13.2.1 *Principle* — Absorption of arsenic in a solution of silver diethyldithiocarbamate involves the reaction:

$$AsH_3 + 6\ Ag(DDTC) = 6\ Ag + 3\ H(DDTC) + As(DDTC)_3$$

The colloidally dispersed silver, purplish-red colour, is measured by photometry (nephelometry) at 540 nm.

13.2.2 *Apparatus*

13.2.2.1 *Evolution and absorption apparatus* — It shall consist of a conical flask *A* of 100 ml capacity for evolution of arsenic, a connecting tube *B* to trap hydrogen sulphide, and absorption tube *C* with a spherical or conical ground glass joint. A spring clip may be used to ensure firm joint between the connecting tube *B* and absorption tube *C* when a spherical joint is used. Suitable forms of apparatus using spherical joint with fritted glass are shown in Fig. 5, 6 and 7

13.2.2.2 *Spectrophotometer or photoelectric absorptiometer* — With filters in the range 520 to 560 nm and 10 mm cells.

NOTE — The glassware should not be rinsed with organic solvents to facilitate drying. Traces of organic matter, especially, acetone may adversely affect the traction between zinc and acid.

13.2.3 *Reagents*

13.2.3.1 *Concentrated hydrochloric acid — see* IS : 265-1976*.

*Specification for hydrochloric acid (*second revision*).

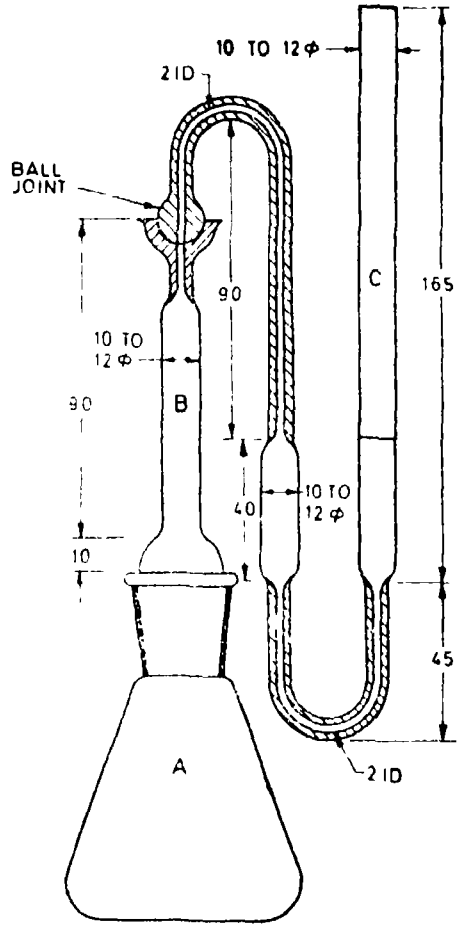


FIG. 5 APPARATUS FOR DETERMINATION OF ARSENIC (SILVER DIETHYLDITHIOCARBAMATE METHOD)

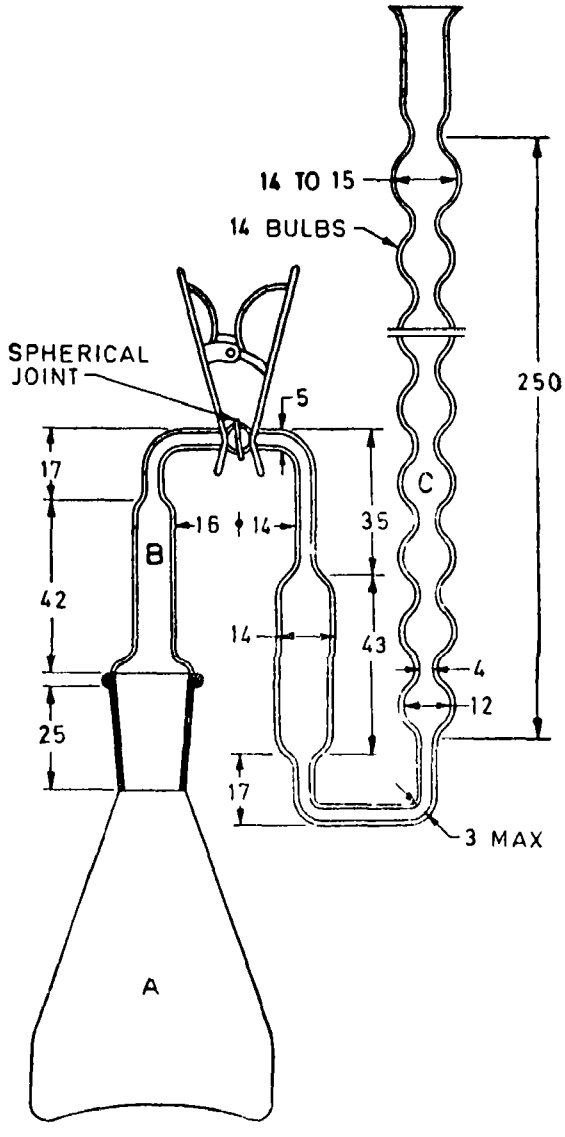
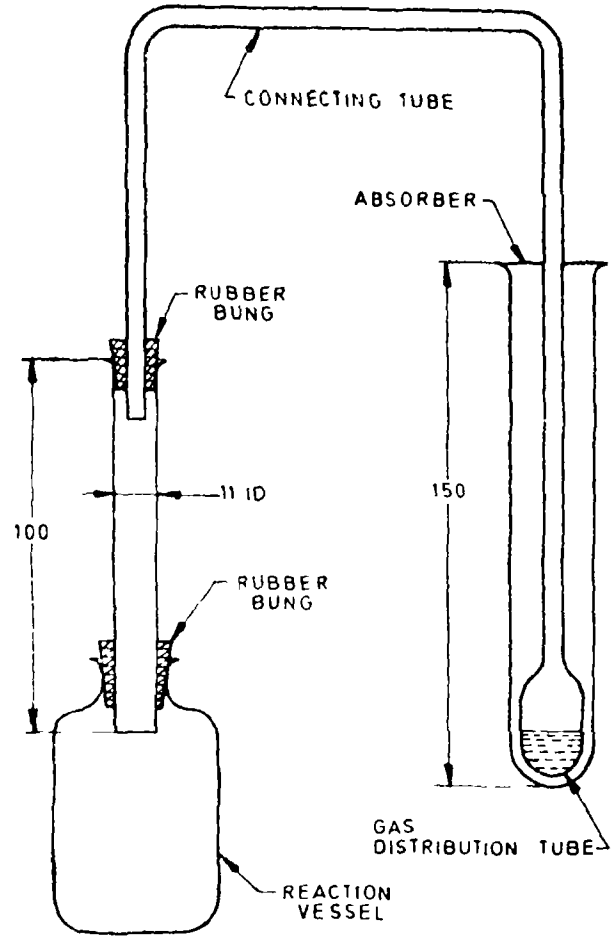


FIG 6 APPARATUS FOR DETERMINATION OF ARSENIC (SILVER DIETHYLDITHIOCARBAMATL METHOD)



All dimension in millimetres.

FIG. 7 APPARATUS FOR DETERMINATION OF ARSENIC (SILVER DIETHYLDITHIOCARBAMATE METHOD)

13.2.3.2 *Potassium iodide solution* — 15 percent (*m/v*).

13.2.3.3 *Stannous chloride solution* — Dissolve 40 g of stannous chloride in 100 ml of hydrochloric acid. Discard this solution if an appreciable deposit forms.

13.2.3.4 *Zinc* — granules of size 0·5 to 1·0 mm.

13.2.3.5 *Silver diethyldithiocarbamate solution* — Dissolve 1 g of silver diethyldithiocarbamate in water-white pyridine and dilute to 200 ml with pyridine. Store the solution in stoppered-glass bottles away from light.

13.2.4 *Procedure*

13.2.4.1 *Preparation of calibration curve* — The curve shall be confirmed every time when a new solution of silver diethyldithiocarbamate is prepared.

13.2.4.2 *Evolution of arsenic* — Transfer to a series of 100 ml conical flasks, aliquots of standard arsenic solution corresponding to 0, 5, 10, 15, 20 and 25 μg of arsenic and proceed as given in (a).

a) Add 10 ml of concentrated hydrochloric acid and dilute to 50 $\pm$ 5 ml with water. Add 2 ml of potassium iodide and stannous chloride solution respectively. Mix well and let it stand for 15 to 20 minutes. Pack lightly the top third of the connecting tube with impregnated absorbent cotton wool and assemble with the absorption tube. Transfer 5·0 ml of silver diethyldithiocarbamate solution to absorption tube *C*. After 15 to 20 minutes, introduce 5 g of zinc granules into the conical flask *A* and quickly reassemble the apparatus. Allow the reaction to proceed for 45 to 60 minutes at room temperature.

b) *Spectrophotometric measurements* — Disconnect the absorption tube and tilt the absorber so that the reagent solution flows back and forth between the absorber so that the reagent solution flows back and forth between the absorber and bulb to disperse the solid contents, if any, and to mix in the solution well. Transfer the solution to a photometric cell and measure its absorbance at the wavelength of maximum absorption, 540 nm, using water as reference liquid.

NOTE — The colour of the dispersion is not very stable for long time and hence absorptiometric (nephelometric) measurement should be made within 2 hours of the development of colour. Care should also be taken to prevent the evaporation of solution as its volume is small.

In the case of fritted glass absorber, raise and lower the connecting tube into the absorber several times to allow the solution to pass through the frit back and forth effecting the dispersal of the red deposit. Let the connecting tube finally drain into the absorber.

10 μg standard shall have an absorption of the order of 0·4.

c) *Plotting of the calibration curve* — Calculate corrected absorbance by subtracting the reading obtained for the solution containing no standard arsenic solution from the observed reading. Plot a graph of corrected absorbance of solution against their arsenic contents.

13.2.4.3 The test solutions shall be prepared so as to contain 1 to 10 μg of arsenic in a solution of 5 0 $\pm$ 0·5 ml volume. Transfer the solution to the conical flask, cool to room temperature, if necessary, and proceed as prescribed in **13.2.4.2**, **13.2.4.2** (a) and **13.2.4.2** (b).

13.2.4.4 *Blank test* — Carry out a blank test as prescribed in **13.2.4.2**, **13.2.4.2** (a) and **13.2.4.2** (b) omitting the sample.

13.2.5 *Calculation* — Calculate the corrected absorbance by subtracting the value obtained for the blank solution from that obtained for the test solution and read from the calibration curve the corresponding mass of arsenic.

$$\text{Arsenic content, mg/kg} = \frac{M_1}{M_2}$$

where

M_1 = mass in μg of arsenic found, and

M_2 = mass in g of sample in the solution tested.

14. DETERMINATION OF LEAD

14.0 Two methods for the determination of lead have been prescribed. Any of these may be used for routine purposes. However, for referee purposes, the atomic absorption spectrophotometric method should be used.

14.1 Atomic Absorption Spectrophotometric Method

14.1.1 *Principle* - Organic matter is digested and lead released co-precipitates with strontium sulphate, soluble sulphate salts are decanted and precipitate is converted to carbonate salt, dissolved in acid and determined by atomic absorption at 217 or 283·3 nm.

14.1.2 *Apparatus*

14.1.2.1 *Atomic absorption spectrophotometer*

14.1.2.2 *Stirring motor* — with eccentric coupling for stirring centrifuge tubes.

14.1.3 *Reagents*

14.1.3.1 *Strontium solution* — 2 percent. Dissolve 6 g strontium chloride ($SrCl_2$, $6H_2O$) in 100 ml water.

14.1.3.2 *Ternary acid mixture* — Add 20 ml sulphuric acid to 100 ml water, mix, add 100 ml nitric acid and 40 ml of 70 percent perchloric acid and mix.

14.1.3.3 *Nitric acid* — Add 128 ml redistilled nitric acid to 500-800 ml distilled or deionized water and dilute to 2 litres.

14.1.3.4 *Lead standard solution*

a) *Stock solutions* — 1 000 μg/ml Dissolve 1·598 5 g lead nitrate [Pb (NO_3)$_2$] recrystallized in about 500 ml 1 N nitric acid in a 1 litre volumetric flask and dilute to volume with 1 N nitric acid

b) *Working solution* — Prepare 100 μg Pb/ml by diluting 10 ml stock solution to 100 ml with 1 N nitric acid Dilute 1, 3, 5, 10, 15 and 25 ml aliquots of this solution to 100 ml with 1 N nitric acid (1, 3, 5, 10, 15 and 25 μg Pb/ml respectively).

14.1.4 *Separation of Lead*

14.1.4.1 Accurately weigh sample containing approximately 10 g dry matter and up to 3 μg lead. Place in 500 ml boiling or Kjeldahl flask and add 1 ml of 2 percent strontium solution and several glass beads. Prepare reagent blank and carry through same operations as sample. Add 15 ml ternary acid mixture for each gram dry matter and let stand for approximately up to 2 hours. Heat under or water vacuum manifold system until flask contains only sulphuric acid.

NOTE — Take care to avoid sample loss from foaming when heat is first applied, and when foaming occurs soon after sample chars Remove heat and swirl flask before continuing digestion Add nitric acid, if necessary.

14.1.4.2 Cool, digest for a few minutes (digest should be cool enough to add about 15 ml water safely, but hot enough to boil when water is added) Wash while still hot into a 40 to 50 ml tapered bottom centrifuge tube and swirl Let cool centrifuge for 10 min at 350 g and decant liquid into water beaker (film-like precipitate may be discarded) Dislodge precipitate by vigorously stirring with eccentric-coupled stirring motor. To complete transfer, add 20 ml water and 1 ml 1 N sulphuric acid to original flask and heat Do not omit this step even though it appears transfer was complete in first wash. Wash hot contents of original digestion flask into centrifuge tube containing precipitate Swirl to mix Cool, centrifuge and decant liquid into waste beakers.

14.1.4.3 Dislodge precipitate by stirring vigorously, add 25 ml saturated ammonium carbonate [(NH_4)$_2$ CO_3] solution (about 20 percent) and stir until all the precipitate is dispersed Let stand for 1 hour Centrifuge and decant liquid into waste beaker. Repeat ammonium carbonate treatment

14.1.4.4 After decanting invert centrifuge tube on paper towel and drain all liquid. Add 5 ml 1 N nitric acid (use larger volume 1 N nitric acid in both sample and blank if more than 25 μg Pb is expected), stir vigorously to expel carbon dioxide or use ultrasonic bath for 2 to 3 minutes. Let stand for 30 minutes and centrifuge if precipitate remains.

NOTE — Use same technique for all samples.

14.1.5 *Determination*

14.1.5.1 Set instrument to previously established optimum conditions, using air acetylene oxidizing flame and 217 or 283·3 nm resonant wavelength. Determine absorbance of sample and blank solutions for more than 5 standards, within optimum working range (10 to 80 percent transmittance) before and after sample readings Flush burner with 1 N nitric acid and check zero point between readings. Determine lead from standard curve of absorbance against μg Pb ml

14.1.6 *Calculations*

14.1.6.1 Lead, mg/kg $\frac{M_1 \times V_1}{M_2}$

where

M_1 = mass in μg of lead,

V_1 = volume in ml of 1 N nitric acid used, and

M_2 = mass in g of sample taken for test.

14.2 Visual Comparison Method

14.2.1 *Apparatus*

14.2.1.1 *Nessler cylinders* — 50 ml capacity.

14.2.2 *Reagents*

14.2.2.1 *Acetic acid* — approximately 33 percent (*v/v*).

14.2.2.2 *Dilute ammonium hydroxide* — approximately 4 N.

14.2.2.3 *Potassium cyanide solution* — Dissolve 10 g of potassium cyanide in 90 ml of water, add 2 ml of hydrogen peroxide (20 volume strength), allow to stand for 24 hours and make up to 100 ml with water.

14.2.2.4 *Sodium sulphide solution* — Dissolve 10 g of sodium sulphide ($Na_2S.9H_2O$) in 100 ml of water.

14.2.2.5 *Standard lead solution* — Dissolve 0·160 g of lead nitrate in 5 ml of concentrated nitric acid (conforming to IS : 264-1976*) and dilute to 100 ml in a graduated flask. Again dilute 10 ml of the solution to 1 000 ml. One millilitre of the solution finally obtained contains 0·01 mg of lead (as Pb).

*Specification for nitric acid (*first revision*).

14.2.3 *Procedure* — Dissolve 5 g of the prepared sample (*see* **4**) in water in a Nessler cylinder and add 5 ml of acetic acid. Make the mixture alkaline with dilute ammonium hydroxide and add 1 ml of potassium cyanide solution If turbid, filter. Add two drops of sodium sulphide solution and mix well Carry out a control test in another Nessler cylinder in exactly the same manner but using 1 ml of standard lead solution in place of the prepared sample. Dilute the solution in both the cylinders to 50 ml mark. Compare the colour produced in the two cylinders against a white background.

14.2.3.1 The material shall be taken to have not exceeded the limit specified, if the intensity of colour obtained with the material is not greater than that obtained in the control test

15. DETERMINATION OF COPPER

15.0 Methods — Three methods, namely, the spectrophotometric (*see* **15.1**) gravimetric (*see* **15.2**) and atomic absorption spectrophotometric method (*see* **15.3**) are prescribed for the determination of copper in the material. Whereas the spectrophotometric method or atomic absorption spectrophotometric method should be used for referee purposes, for routine analysis the gravimetric method may be used wherever facilities for spectrophotometric analysis are not available.

15.1 Spectrophotometric Method

15.1.1 *Apparatus*

15.1.1.1 *Spectrophotometer* — of a suitable type.

15.1.2 *Reagents*

15.1.2.1 *Concentrated sulphuric acid*— sp gr 1·84.

15.1.2.2 *Sodium carbonate* — solid.

15.1.2.3 *Concentrated hydrochloric acid* — sp gr 1 16, diluted with an equal volume of water

15.1.2.4 *Citric acid* — solid.

15.1.2.5 *Ammonium hydroxide solution* — sp gr 0·90.

15.1.2.6 *Sodium diethyldithiocarbamate solution* — 0·1 percent (*m/v*) aqueous

15.1.2.7 *Carbon tetrachloride* — re-distilled.

15.1.2.8 *Sodium sulphate* — anhydrous.

15.1.2.9 *Concentrated nitric acid* — sp gr 1·42 diluted with an equal volume of water

15.1.2.10 *Standard copper solution* — weigh accurately 0·100 0 g of pure copper turning, carefully dissolve in minimum amount of nitric acid, cool and dilute to one litre. Pipette 10 ml of this solution into a 130 *ml volumetric flask dilute to the mark. This solution contains 10* μg of copper per millilitre.

15.1.3 *Procedure*

15.1.3.1 *Preparation of test solution* — Weigh accurately about 20 0 g of the prepared sample (*see* **4.1**) in a platinum dish and add to it 2 ml of concentrated sulphuric acid. Heat the dish gently over a Bunsen burner until charring is complete and ash the residue in a muffle furnace at 550 to 600°C. Cool the dish, add to the ash about 2 g of sodium carbonate and fuse the contents of the dish for 10 minutes at 900°C. Cool the dish and dissolve the fused matter in the minimum amount of hydrochloric acid, covering the dish with a watch-glass to avoid loss by spattering. Heat the dish until solution is complete Cool the dish and make up the solution to 100 ml in a volumetric flask with water.

15.1.3.2 Transfer 10 ml of the test solution to a separating funnel by means of a pipette. Add one gram of citric acid to the test solution and dissolve it by shaking. Make the resulting solution alkaline to litmus paper by adding ammonium hydroxide solution in small quantities. Add to this alkaline solution, 5 ml of sodium diethyldithiocarbamate solution, shake thoroughly and extract with 5 ml portions of carbon tetrachloride until the final extract is colourless (about four extractions are usually adequate). Dry the combined extracts by shaking thoroughly with anhydrous sodium sulphate. Filter the dry extract and wash the filter paper with carbon tetrachloride Make up the volume of the filtrate to 25 ml with carbon tetrachloride and measure the absorption at 437 mμ by means of the spectrophotometer.

15.1.3.3 Prepare a series of standard colour solutions, using different volumes of standard copper solution instead of 10 ml of the test solution and proceeding as described under **15.1.3.2**. This series should cover the concentration of the colour solutions prepared from the test solution. Measure the absorption of each of the colour solutions in the series.

15.1.3.4 Carry out blank determinations on the water and the reagents used in the preparation of the standard colour solutions (*see* **15.1.3.3**) and colour solution from the material (*see* **15.1.3.1** and **15.1.3.2**). If the values so obtained are of any significance, correct the respective values observed for the series of standard colour solution (*see* **15.1.3.3**) and the test solution (*see* **15.1.3.2**) accordingly.

15.1.3.5 Plot a curve using the corrected values for absorption and the corresponding concentration of copper in micrograms present in each standard colour solution in the series From this curve obtain the mass in micrograms of copper present in 10 ml of the test solution (*see* **15.1.3.1**).

15.1.4 *Calculation*

Copper (as Cu) content of the material, parts per million $= \frac{10\,M}{W}$

where

M = mass in μg of copper present in 10 ml of the test solution (*see* **15.1.3.5**), and

W = mass in g of the material taken for the test.

15.2 Gravimetric Method

15.2.1 *Reagents* — The following reagents are required. The reagents shall be free from traces of copper.

15.2.1.1 *Concentrated sulphuric acid* — sp gr 1·84.

15.2.1.2 *Sodium carbonate* — solid.

15.2.1.3 *Hydrochloric acid* — concentrated hydrochloric acid of sp gr 1·16 with an equal volume of water.

15.2.1.4 *Sodium hydroxide solution* — approximately 2 N.

15.2.1.5 *Sodium acetate* — crystalline.

15.2.1.6 *Glacial acetic acid*

15.2.1.7 *Salicylaldoxime solution* — Dissolve one gram of salicylaldoxime in 5 ml of rectified spirit without the aid of heat. Gently, pour this solution into 95 ml of water at 80°C. The oxime partially separates out in the form of a fine oil suspension, but quickly redissolves. Avoid shaking at this stage as shaking helps the small droplets of the oxime grow. When the solution becomes clear, shake it for some time and filter. Use the filtrate.

15.2.1.8 *Ferrous chloride solution* — about 5 percent (*m v*).

15.2.2 *Procedure*

15.2.2.1 Proceed as desired under **15.1.3.1**, but do not make up the volume to 100 ml.

15.2.2.2 To the whole of the solution so obtained, add sodium hydroxide solution until a lasting precipitate is formed. Add one gram of sodium acetate and 10 ml of glacial acetic acid, and stir until the precipitate redissolves. Dilute the resulting solution to about 100 ml with water. Add to this dilute solution a bare excess of salicylaldoxime solution to precipitate all the copper present in the solution. Coagulate the precipitate by stirring with a glass rod and allow to settle.

Test the supernatant liquid for completeness of precipitation by adding several drops of salicylaldoxime solution. (A considerable excess of salicylaldoxime should be avoided, as the precipitate has to be washed free from it before drying. Otherwise the precipitate would be visibly decomposed during drying.) Filter the precipitate through a tared No. 3 Gooch crucible and wash it with cold water until the filtrate ceases to give any colour with ferrous chloride solution. During washing, take care that the precipitate always remains moist. Finally, wash the precipitate twice again with water, and then dry it as far as possible by suction. Dry the crucible with contents to constant weight at 105 to 110°C. Cool in a desiccator and weigh. Find the mass of the dried precipitate.

15.2.3 *Calculation*

$$\text{Copper (as Cu), mg/kg} = \frac{189\,200\,m}{M}$$

where

m = mass in g of the dried precipitate as determined under **15.2.2.2**, and

M = mass in g of the material taken for the test.

15.3 Atomic Absorption Spectrophotometric Method

15.3.1 *Principle*

15.3.1.1 Samples are wet ashed and after dilution are aspirated into C_2H_2 flame. Radiation at 324·7 nm from copper hollow cathode lamp is passed through flame. Attenuation is measured in spectrophotometer calibrated with known concentrations of copper in the presence of matrix similar to that of samples which avoids interference from elements such as Na and K. Concentration range is 5-100 mg/kg depending on the sensitivity of the instrument working range is 0 2-10 µg/ml. Recommended upper limit is that which gives absorbance about 0·4.

15.3.2 *Apparatus*

15.3.2.1 *Atomic absorption spectrophotometer*

15.3.3 *Reagents*

15.3.3.1 *Copper standard solution* — 1 000 µg ml. Dissolve 1·000 g of 99·99 percent copper in 20 ml nitric acid, cool and dilute to 1 litre with water.

15.3.3.2 *Matrix standard solution* — Prepare solutions containing 0, 0·2, 0·4, 0·8, 1·6, 2·0, 4·0, 8·0 and 10 µg Cu/ml and major metal matrix components: (a) for 3 g sample to contain 180 µg Ca, 100 µg Mg and 40 µg Al with final concentrations of 8 percent (*v/v*) perchloric acid and (b) for 6 g sample to contain 7 000 µg K, 70 µg Na, 700 µg Mg, and 130 µg Ca/ml with final nitric acid concentrates of 1:9.

15.3.4 *Preparation of Calibration Curve*

15.3.4.1 With Cu hollow cathode tube in position, energized, and stabilized, locate wavelength setting that gives maximum response to radiation at 324 7 nm. Wash combustion chamber and burners head with nitric acid (1:1). Light burner, let it reach thermal equilibrium and zero instrument while aspirating water Aspirate 10 μg Cu/ml standard solution and adjust burner height, air and fuel pressures and flow rates, aspiration rate of solution, and position of capillary to obtain maximum response. Adjust slit setting and gain to obtain optimum signal-to-noise ratio. Recalibrate when any of these parameters change. Absorbance should be about 0 32 for 10 μg Cu/ml. Scale expansion is required to obtain appreciable readings for copper up to 2 μg/ml.

15.3.4.2 Aspirate 10 μg/ml standard enough times to establish that absorbance reading is not drifting. Record 6 readings and calculate standard deviation (σ) $(x - y) \times 0{\cdot}40$ where x and y are maximum and minimum readings respectively and 0·40 is factor to convert range of 6 values to σ

15.3.4.3 Beginning with solution containing 0 μg/ml Cu, aspirate each matrix standard solution and record absorbance If value for 10 μg/ml solution differs from average of 6 values used to calculate σ by more than 0 01 x (average of the 6 values), repeat measurements if these determinations indicate drift, determine cause (for example, deposits in burner or clogged capillary), correct it, add repeat calibration. Plot absorbance against μg metal/ml

15.3.5 *Determination*

15.3.5.1 Select samples mass to give solution containing 0·05 to 10 μg Cu/ml.

15.3.5.2 *Wet ashing* — Accurately weigh sample into 400 ml beaker, add 100 ml nitric acid and swirl. Cover and let react for 10 minutes, then place on hot plate Evaporate to near dryness and cool Add 50 ml nitric acid and 10 ml perchloric acid. Continue evaporation to obtain clear solution. Transfer to 50-ml volumetric flask and dilute to volume with water (insoluble potassium perchlorate, which settles to bottom of flask and does not interfere) Prepare reagent blank containing same amounts of acids taken from same lots evaporated as above.

15.3.5.3 *Photometery* — Aspirate sample and blank solution and record absorbance Measure absorbance of matrix standard solution containing 10 μg/ml If this value differs from value of the average of the 6 values used to calculate σ by more than 2 σ, repeat measurement. If these values indicate drift, determine cause, correct it, and repeat calibration of sample and blank readings

15.3.5.4 *Calculations* — Correct readings of sample solution for blank. Convert corrected absorbance to μg/ml from calibration curve.

$$\text{Copper, mg/kg} = \frac{C \times v}{W'}$$

where

C = μg metal/ml from curve,

v = final volume sample solution (50 ml), and

W' = mass in g of sample.

16. DETERMINATION OF ZINC

16.0 Two methods for the determination of zinc have been prescribed. Any of these may be used

16.1 Spectrophotometric/Colorimetric Method

16.1.0 *Principle* — This method involves wet oxidation of the material, elimination of lead, copper, cadmium, bismuth, antimony, tin, mercury and silver as sulphides, using copper sulphate solution as scavanger agent, simultaneous elimination of cobalt and nickel by extracting metal complexes of α-nitroso β-naphthol and dimethyl glyoxime respectively with chloroform, extraction of the zinc dithizonate with carbon tetrachloride, transfer of zinc to dilute hydrochloric acid, and final extraction of zinc dithizonate for colour measurement.

16.1.1 *Apparatus*

16.1.1.1 *Spectrophotometer* — of a suitable type.

16.1.2 *Reagents*

16.1.2.1 *Water* — Re-distil the water from all-glass Pyrex or equivalent glassware, which is scrupulously cleaned with hot concentrated nitric acid.

16.1.2.2 *Concentrated nitric acid* — re-distilled end of sp gr 1·42.

16.1.2.3 *Concentrated sulphuric acid* — sp gr 1·84.

16.1.2.4 *Perchloric acid* ($HClO_4$)

16.1.2.5 *Methyl red indicator solution* — one percent (m/v), aqueous.

16.1.2.6 *Copper sulphate solution* — Dissolve 8 g of copper sulphate ($CuSO_4 . 5H_2O$) in water and dilute to one litre One millilitre of this solution contains 2 mg of copper (Cu).

16.1.2.7 *Ammonium hydroxide solution* — re-distilled and of sp gr 0·90

16.1.2.8 *Concentrated hydrochloric acid* — sp gr 1 16.

16.1.2.9 *Hydrogen sulphide gas* — from the generator, passed through a wash bottle containing water.

16.1.2.10 *Dilute hydrochloric acid* — containing 5 percent $\left(\frac{m}{v}\right)$.

16.1.2.11 *Bromine water* — saturated.

16.1.2.12 *Phenol red indicator solution* — 0·04 percent (*m/v*). Dissolve 0·1 g of phenol red in 28·2 ml of 0·01 N sodium hydroxide solution and dilute to 250 ml with water.

16.1.2.13 *Hydrochloric acid (1 . 1)* — dilute concentrated hydrochloric acid with an equal volume of water

16.1.2.14 *Dithizone (diphenylthiocarbazone) solution* — Dissolve 0·05 g of dithizone in 2 ml of ammonium hydroxide solution and 100 ml of water, and extract repeatedly with carbon tetrachloride until solvent layer is clear and of bright green colour. Discard the solvent layer and filter the aqueous portion through a washed ashless paper (This solution is best prepared as needed since it is only moderately stable, even when kept in the dark and under refrigeration).

16.1.2.15 *Carbon tetrachloride* — re-distilled.

16.1.2.16 *Chloroform* — re-distilled.

16.1.2.17 *Ammonium citrate solution* — Dissolve 225 g of ammonium citrate [(NH_4)$_2$ $HC_6H_5O_7$] in water, make alkaline to phenol red with ammonium hydroxide (*p*H 7·4 first distinct colour change) and add 75 ml in excess. Dilute to 2 litres Extract this solution immediately before use as given below

Add to the ammonium citrate solution (*see* above) a slight excess of dithizone solution and extract with carbon tetrachloride until the solvent layer is clear bright green. Remove the excess of dithizone by repeated extraction with chloroform, and finally extract once more with carbon tetrachloride. (It is essential that excess dithizone be entirely removed, as otherwise zinc will be lost during elimination of cobalt and nickel).

16.1.2.18 *Dimethyl glyoxime solution* — Dissolve 2 g of dimethyl glyoxime in 10 ml of ammonium hydroxide solution and 200 to 300 ml of water, filter and dilute to one litre.

16.1.2.19 *α-Nitroso-β-naphthol solution* — Dissolve 0·25 g of α-nitroso-β-naphthol in chloroform and dilute with chloroform to 500 ml.

16.1.2.20 *Hydrochloric acid* — 0·04 N approximate, prepared from concentrated hydrochloric acid

16.1.2.21 *Stock solution of zinc* — Dissolve exactly 0·500 g of pure granulated zinc in a slight excess of dilute hydrochloric acid and dilute to 1 000 ml.

16.1.2.22 *Standard solution of zinc* — At the time of the experiment, dilute 10 ml of the stock solution of zinc (*see* **16.1.2.21**) to 1 000 ml with hydrochloric acid (0 04 N). This solution contains 5 μg of zinc per millilitre.

16.1.3 *Procedure*

16.1.3.1 *Preparation of material* — Accurately weigh into an Erlenmeyer flask of suitable size a quantity of the prepared sample (*see* **4.1**) not exceeding 25 g, estimated to contain 25 to 100 μg of zinc. Add to the prepared sample concentrated nitric acid and heat cautiously until the first vigorous reaction subsides somewhat, then add 2 to 5 ml of concentrated sulphuric acid. Continue the heating, adding, if necessary, more of concentrated nitric acid in small increments, to prevent charring until fumes of sulphur trioxide are evolved and the solution becomes clear and almost colourless. Add 0·5 ml of perchloric acid and continue heating until it has been completely removed. Allow the solution to cool and dilute it to about 40 ml. (If necessary equipment is available, wet oxidation and subsequent sulphide separation may be advantageously carried out in a small Kjeldahl flask).

16.1.3.2 *Separation of sulphide group* — To the sulphuric acid solution of the material (*see* **16.1.3.1**) contained in the Erlenmeyer flask add 2 drops of methyl red indicator solution and one millilitre of copper sulphate solution, and neutralize with ammonium hydroxide solution. Add sufficient quantity of concentrated hydrochloric acid to make the solution about 0·15 N with respect to this acid (about 0·75 ml excess in 50 ml of solution is satisfactory). The *p*H of the solution at this point should be 1·9 to 2·1 when measured with glass electrode. Pass a stream of hydrogen sulphide gas into the solution until precipitation is complete. Filter the contents of the flask through a fine textured filter paper (Whatman No. 42 or an equivalent) that has been previously fitted into the funnel and washed first with dilute hydrochloric acid, and then with water. Collect the filtrate in a beaker and wash the flask and the filter with 3 or 4 small portions of water. Boil the filtrate gently until the colour of hydrogen sulphide can no longer be detected, then add 5 ml of bromine water and continue boiling until bromine has been expelled. Allow the solution to cool, neutralize with ammonium hydroxide solution using phenol red indicator solution, and then make it slightly acidic with hydrochloric acid (1 : 1) by adding an excess of 0·2 ml. Dilute the resultant solution in a volumetric flask to contain 0·2 to 1·0 g of zinc per ml.

16.1.3.3 *Elimination of nickel and cobalt* — Transfer a 20 ml aliquot of the solution (*see* **16.1.3.2**) to a separating funnel. Add to it 5 ml of ammonium citrate solution, 2 ml of dimethyl glyoxime solution and 10 ml of α-nitroso-β-naphthol solution, and shake the contents of the separating funnel for 2 minutes. Discard the layer of chloroform and extract the aqueous layer with 10 ml of chloroform to remove residual α-nitroso-β-naphthol. Discard the chloroform layer.

16.1.3.4 *Isolation and estimation of zinc*

a) To the aqueous layer obtained after eliminating nickel and cobalt (*see* **16.1.3.3**), which at this point has a *p*H of 8·0 to 8·2, add 2·0 ml of the dithizone solution and 10 ml of carbon tetrachloride, and shake after 2 minutes. Allow the layers to separate and remove the aqueous layer as completely as possible withdrawing it by means of a pipette attached to the vacuum line. Wash down the sides of the separating funnel with about 25 ml of water and without shaking again draw off the aqueous layer. Add 25 ml of hydrochloric acid (0·04 N) to the carbon tetrachloride layer in the separating funnel and shake for one minute to transfer the zinc to the acid-aqueous layer. Drain off and discard the carbon tetrachloride layer; being careful to dislodge and remove the drop that usually floats on the surface. To the acid-aqueous layer add 5·0 ml of ammonium citrate solution and 10·0 ml of carbon tetrachloride (*p*H of the solution at this point is 8·8 to 9·0). Determine the volume of dithizone solution to be added as given in (b).

b) Pipette 4·0 ml standard solution of zinc into a separating funnel, add to it 21 ml of hydrochloric acid (0·04 N) from a burette, 5 ml of ammonium citrate solution and 10·0 ml of carbon tetrachloride, and then add dithizone solution in 0·1 ml increments, shaking briefly after each addition until a faint yellow colour in the aqueous layer indicates a bare excess of the reagent. Note the total volume of dithizone solution added. Multiply this volume by 1·5.

c) Add the volume [*see* **16.1.3.4** (b)] of dithizone solution to the solution contained in the separating funnel (*see* **16.1.3.4**) and shake it for two minutes. Pipette 5·0 ml of the cabon tetrachloride layer and transfer it to the spectrophotometer cell. Dilute the solution with 10·0 ml of carbon tetrachloride, mix and determine its spectral transmission at 540 mμ.

Dilution may be made in a clean and dry test-tube, if the design of the cell does not permit mixing directly.

d) Pipette into a series of separating funnels, 0, 1, 2, 3 and 4 ml of standard solution of zinc and add the necessary volume of hydrochloric acid (0·04 N) to make 25 ml. Add into each separator 5·0 ml of ammonium citrate solution and the calculated volume of dithizone solution [*see* **16.1.3.4** (b)]. Shake the separating funnels for two minutes. From the first separating funnel pipette out 5·0 ml of the carbon tetrachloride layer and transfer it to the spectrophotometer cell. Dilute the solution with 10·0 ml of carbon tetrachloride, mix and determine its spectral transmission at 540 mμ. Proceed in the same manner with the solutions contained in other separating funnels.

e) Plot the transmittance on logarithmic scale obtained for each of the series of separating funnels (*see* **16.1.2.4**) against concentration of zinc in micrograms present in 25 ml of the diluted standard zinc solution in the particular separating funnel and draw a smooth curve through the points. (Intercept of the curve may vary from day to day, depending on the actual concentration of dithizone used in the final extraction, but the slope of the curve should remain essentially same.) From the curve obtain the mass of zinc in micrograms present in 25 ml of the acid aqueous layer (for final extraction of zinc from the material, *see* **16.1.3.4**).

16.1.4 *Calculation*

16.1.4.1 Zinc, mg/kg = $\frac{M \times V}{20 \times W}$,

where

M = mass in g of zinc present in 25 ml of the acid aqueous layer prepared from the material [*see* **16.1.3.4** (d)],

V = volume in ml of the solution made to contain 0·2 to 1·0 g of zinc per ml prepared after eliminating the sulphide group from the material (*see* **16.1.3.2**), and

W = mass in g of the prepared sample taken for the experiment (*see* **16.1.3.1**).

16.2 Atomic Absorption Spectrometric Method

16.2.1 *Principle*

16.2.1.1 Decomposition of organic matter by the dry or wet method and determination of the Zn^{2+} cation by atomic absorption spectrometry.

16.2.2 *Reagents* — All reagents shall be especially free of zinc. The water to be used shall have been distilled twice in an apparatus of borosilicate glass, or shall be water of at least equivalent purity.

16.2.2.1 Sulphuric acid, d_{20} = 1·83 g ml.

16.2.2.2 Nitric acid, d_{20} = 1·38 g/ml.

16.2.2.3 Perchloric acid, d_{20} = 1·67 g/ml.

16.2.2.4 Hydrochloric acid, d_{20} = 1·19 g/ml.

16.2.2.5 *Hydrochloric acid, 1 + 1 solution* — Mix one volume of the hydrochloric acid (*see* **6.2.2.4**) with one volume of water.

16.2.2.6 Hydrochloric acid (HCl) = 0·1 mol/l*.

16.2.2.7 *Zinc, standard solution corresponding to 1 g of zinc per litre* — In a 1 000 ml volumetric flask, dissolve 4·396 6 g of zinc sulphate heptahydrate ($ZnSO_4, 7H_2O$) in water and make up to the mark. Store the solution in a bottle of borosilicate glass fitted with a glass stopper.

16.2.3 *Apparatus* — Usual laboratory equipment, not otherwise specified, and the following.

16.2.3.1 *Round bottom flasks* — of capacity 1 000 ml.

16.2.3.2 *Platinum or quartz dishes* — of diameter 70 mm.

16.2.3.3 *Volumetric flasks* — of capacity 50 ml.

16.2.3.4 *Pipettes*

16.2.3.5 *Funnels and ashless filter paper*

16.2.3.6 *Boiling water bath*

16.2.3.7 *Electrically heated muffle furnace* — Capable of being controlled at 525 ± 25°C.

16.2.3.8 *Atomic absorption spectrometer* — Fitted with an air acetylene burner, for measurement at a wavelength of 213·8 nm.

16.2.3.9 *Infra-red lamp*

16.2.3.10 *Analytical balance*

16.2.4 *Procedure*

16.2.4.0 Weigh to the nearest 0·01 g, 5 to 10 g of the test sample (*see* **4.1**) and decompose by the dry or wet methods.

16.2.4.1 *Decomposition by dry method* — Introduce the test portion (*see* **16.2.4.0**) into one of the dishes (*see* **16.2.3.2**) and place it in the boiling water bath. Evaporate to dryness. Burn the organic matter by means of the infra-red lamp or, if such a lamp is not available, by

*Hitherto expressed as approximately 0·1 N solution.

means of Bunsen burner; continue the decomposition in the muffle furnace controlled at 525 ± 25°C until white ashes are obtained. Dissolve the ashes in 1 to 2 ml of the dilute hydrochloric acid (*see* **16.2.2.5**), add about 20 ml of water and place in the boiling water bath until completely evaporated. Add 20 ml of the hydrochloric acid solution and heat on the water bath for about 5 min. Filter through an ashless filter paper. Collect the filtrate in one of the 50 ml volumetric flasks (*see* **16.2.3**) and rinse the dish and filter paper repeatedly with 5 to 10 ml of the hydrochloric acid solution (*see* **16.2.2.6**). Cool and dilute to the mark with the same hydrochloric acid solution.

16.2.4.2 *Blank test* — Carry out a blank test, replacing the test portion by 10 ml of water, and proceeding as described in **16.2.4.1**.

16.2.4.3 *Decomposition by wet method* — Introduce the test portion into one of the round bottom flasks. Add 10 ml of nitric acid and 5 ml of sulphuric acid together with some glass beads. Place the flask containing the mixture on the digestion rack and heat, cautiously to avoid excessive frothing. If necessary, interrupt heating and begin again only when vigorous frothing has ceased. As soon as possible, bring the liquid to boil and continue boiling until it begins to turn brown. Then add 1 to 2 ml of nitric acid drop by drop. Boil again after every addition, but avoiding vigorous heating. Care shall be taken to always have some nitric acid in the mixture as indicated by the presence of nitrous vapours. Stop the addition of nitric acid when the solution no longer turns brown on addition of the acid. Continue heating until white fumes appear, indicating a high concentration of sulphuric acid and a reduction in nitric acid. If the solution turns brown again, continue the addition of nitric acid and repeat the operation described above until browning ceases. Allow the solution to cool. The absence of colour or the presence of a light green or yellow colour indicates that the digestion is complete. Carefully add 15 ml of water to the cold solution, and boil until white fumes appear. Repeat this operation twice more.

When decomposition is terminated, dilute the solution with a few millilitres of water and filter through an ashless filter paper Collect the filtrate in one of the 50 ml volumetric flasks and rinse the flask and the filter paper with a few millilitres of water, collecting the rinsings in the volumetric flask. Shake, cool, dilute to the mark and homogenize by shaking.

16.2.4.4 *Blank test* — Carry out a blank test, replacing the test portion by 10 ml of water, and proceeding as described in **16.2.4.3**.

NOTE — The blank test is unnecessary if the absence of zinc in the reagents for decomposition has been verified.

16.2.4.5 *Determination*

a) *Sample decomposed by dry method*

i) *Preparation of the calibration curve* — Dilute the standard zinc solution with the hydrochloric acid (*see* **16.2.2.6**) to obtain four solutions containing 0·25 — 0·5 — 1 and 1·5 mg of zinc per litre. Aspirate each of these solutions into the flame of the spectrometer, at a rate of approximately 4 ml/min. Record the corresponding values of absorbance and draw the calibration curve.

ii) *Spectrometric measurement on the test solution* — Aspirate the test solution (*see* **16.2.4.1**) into the flame of the spectrometer at a rate of approximately 4 ml/min. Record the absorbance.

iii) *Spectrometric measurement on the blank test solution* — Aspirate the blank test solution (*see* **16.2.4.2**) into the flame of the spectrometer, and record the absorbance. The absorbance shall be less than or equal to 0·002. Subtract the absorbance of the blank test solution from that of the test solution (*see* **16.2.4.1**).

b) *Sample decomposed by wet method*

i) *Preparation of the calibration curve* — Dilute the standard zinc solution with water to obtain four solutions containing 2·5 — 5 — 10 and 15 mg of zinc per litre into a series of four 50 ml volumetric flasks, place 5 ml of each of these solution, add 30 to 35 ml of water, and then 5 ml of the sulphuric acid. Shake, cool, dilute to the mark with water and homogenize by shaking. These solutions contain respectively 0·25 — 0·5 — 1 and 1·5 mg of zinc per litre. Aspirate each of these solutions into the flame of the spectrometer at a rate of approximately 4 ml/min. Record the corresponding values of absorbance and draw the calibration curve.

ii) *Spectrometric measurement on the test solution* — Aspirate the test solution (*see* **16.2.4.3**) into the flame of the spectrometer at a rate of approximately 4 ml/min. Record the absorbance*.

iii) *Spectrometric measurement on the blank test solution* — Aspirate the blank test solution (*see* **16.2.1.1**) into the flame of the spectrometer and record the absorbance. The absorbance shall be less than or equal to 0·002. Subtract the absorbance of the blank test solution from that of the test solution (*see* **16.2.4.3**).

*If the absorbance of the test solution exceeds that of the most concentrated calibration solution, suitably dilute the test solution.

16.2.4.6 *Expression of results*

a) *Calculation*

$$\text{Zinc, mg/kg} = \frac{c \times 50}{m}$$

where

c = concentration of zinc in milligrams per litre read from the calibration curve, and

m = mass in grams of the test portion.

NOTE — If it is desired to express the zinc content relative to the dry product, take this into account in the calculation.

b) *Repeatability*

i) The difference between the results of two determinations carried out simultaneously or in a rapid succession by the same analyst, on the same sample, shall not exceed 10 percent (relative).

17. DETERMINATION OF TIN

17.0 Two methods for the determination of tin have been prescribed. Any of these may be used.

17.1 Gravimetric Method

17.1.1 *Reagents*

17.1.1.1 *Concentrated nitric acid* — sp gr 1·42.

17.1.1.2 *Concentrated sulphuric acid* — sp gr 1·84.

17.1.1.3 *Hydrogen peroxide solution* — 30 percent by mass.

17.1.1.4 *Ammonium oxalate solution* — saturated, aqueous.

17.1.1.5 *Ammonium hydroxide solution* — sp gr 0·90.

17.1.1.6 *Concentrated hydrochloric acid* — sp gr 1·16.

17.1.1.7 *Dilute sulphuric acid* — 1·3 by volume.

17.1.1.8 *Hydrogen sulphide gas* — from generator, passed through a wash bottle containing water.

17.1.1.9 *Wash solution* — Mix 100 ml of saturated ammonium acetate solution with 50 ml of glacial acetic acid and 850 ml of water.

17.1.1.10 *Ammonium polysulphide solution* — Pass hydrogen sulphide gas through 200 ml of ammonium hydroxide solution, contained in a bottle immersed in ice-cold water, until no more gas is absorbed. Add to it 200 ml of ammonium hydroxide solution, and dilute with water to make 1 000 ml. Then add 25 g of flowers of sulphur to this solution and keep for several hours to digest the sulphur and then filter.

17.1.1.11 *Dilute acetic acid* — Dilute one volume of glacial acetic acid with 9 volumes of water.

17.1.2 *Procedure*

17.1.2.1 *Wet oxidation* — Weigh accurately 50 to 100 g of the prepared sample (*see* **4.1**) and place it in a 800-ml Kjeldahl flask. Add to it 25 to 50 ml of concentrated nitric acid and then cautiously add 20 ml of concentrated sulphuric acid. Place the flask on an asbestos mat having a 5 cm diameter hole in the centre. Warm the flask slightly and discontinue heating if foaming becomes excessive. When the reaction subsides, heat the flask cautiously and rotate it from time to time to prevent caking of the material at the bottom of the flask which is exposed to the flame. Maintain the oxidizing conditions in the flask by cautiously adding small quantities of concentrated nitric acid whenever the mixture turns brown or darkens. Continue digestion until all the organic matter is destroyed and sulphur trioxide fumes are copiously evolved. (The final solution should be colourless, or at the most light straw coloured. A persistent yellow colour in the liquid may be discharged by the addition of hydrogen peroxide solution and heating again). Slightly cool the flask and add 75 ml of water and 25 ml of ammonium oxalate solution to assist in expelling oxides of nitrogen from the solution. Evaporate the solution again to a point where fumes of sulphur trioxide appear in the neck of the flask and then cool it.

17.1.2.2 *Determination* — Add 200 ml of water to the contents of the Kjeldahl flask (*see* **17.1.2.1**) and transfer the contents to a 600 ml beaker. Rinse the Kjeldahl flask three times with boiling water, transfer the washings to the beaker to make a total volume at about 400 ml. Cool the contents of the beaker, add ammonium hydroxide solution until the contents are just alkaline, then add 20 ml of either concentrated hydrochloric acid or dilute sulphuric acid. Cover the beaker with a watch glass, heat the solution contained in the beaker to about 95°C and pass slow stream of hydrogen sulphide gas through the solution for one hour. Digest this mixture at about 95°C for one hour and allow it to stand for 30 minutes. Filter the contents of the beaker through a quantitative filter paper and wash the residue of stannous sulphide on the filter alternately with three portions each of wash solution and hot water. Transfer the residue, along with the filter paper, to a 50-ml beaker, add 10 to 20 ml of ammonium polysulphide solution, heat the

contents of the beaker to boiling and filter by decantation. Add 10 ml of ammonium polysulphide solution to contents of the beaker, just bring to boil and filter again in the same manner. Repeat this once more. Finally wash the filter with hot water. Acidify the combined filtrate and washings with dilute acetic acid, gently boil for one hour and allow to stand overnight. Filter the resulting mixture through a double 11-cm ashless filter paper. Wash the filter alternately with two portions each of the wash solution and hot water. Transfer the residue along with the filter paper to a tared porcelain crucible and dry it thoroughly in an air-oven. Carefully ignite the filter paper, using a Bunsen flame, and incinerate the contents to convert the sulphide to oxide. Then partly cover the crucible and heat strongly over a large Bunsen or Maker burner. (The sulphide shall be gently roasted to the oxide, which may then be heated to a high temperature without loss by volatilization.) Cool the crucible in a desiccator, weigh and find the weight of the residue. Repeat heating strongly cooling and weighing until the weight of crucible and its contents remain constant.

17.1.3 *Calculation*

17.1.3.1 Tin (as Sn), mg/kg $\quad \frac{787\,700\,M_1}{M_2}$

where

M_1 = mass in g of the residue (SnO_2) left in the porcelain crucible (*see* **17.1.2.2**), and

M_2 = mass in g of the prepared sample taken for the experiment.

17.2 Volumetric Method

17.2.1 *Reagents*

17.2.1.1 *Air-free wash solution* — Dissolve 20 g sodium bicarbonate in two litres boiled water and add 40 ml hydrochloric acid freshly prepared.

17.2.1.2 *Iodine standard solution* — 0·01 N. Standardize solution frequently against tin standard solution adding asbestos mat and proceeding as in **17.2.2**, omitting precipitation with hydrogen sulphide and boiling with hydrochloric acid and potassium chlorate. Amount of tin in solution used for standardization should equal approximately that contained in sample under examination.

17.2.1.3 *Tin standard solution* — 1 mg/ml. Dissolve 1 g tin in about 500 ml hydrochloric acid and dilute to 1 litre with water.

17.2.1.4 *Sheet aluminium* — About 300 gauge, tin-free.

17.2.1.5 *Starch indicator* — Dilute 1 g soluble starch to 200 ml.

17.2.2 *Procedure*

17.2.2.1 Carry out the wet oxidation as given in **17.1.2.1.**

17.2.2.2 Add 200 ml of water to the contents of the Kjeldahl flask (*see* **17.1.2.1**) and transfer the contents to a 600 ml beaker. Rinse the Kjeldahl flask three times with boiling water, transfer the washings to the beaker to make a total volume of about 400 ml. Cool the contents of the beaker, add ammonium hydroxide solution until the contents are just alkaline, then add 20 ml of either concentrated hydrochloric acid or dilute sulphuric acid. Cover the beaker with a watch glass, heat the solution contained in the beaker to about 95°C and pass slow stream of hydrogen sulphide gas through the solution for one hour. Digest this mixture at about 95°C for one hour and allow it to stand for 30 minutes. Filter through asbestos in cold crucible using suction. Wash precipitate of stannous sulphide few times with water and transfer detachable bottom and asbestos pad to a 300 ml Erlenmeyer flask. Remove all traces of precipitate from inside of crucible, using a jet of hot water and policemen, and using a minimum amount of water for washing.

17.2.2.3 Add 100 ml hydrochloric acid and 0·5 g potassium chlorate to flask. Boil for about 15 minutes, making about 4 more additions of smaller amounts of potassium chlorate as chlorine is boiled out of solution. Wash particles of potassium chlorate down from neck of flask with water and finally boil to remove chlorine. Add about 1 g of sheet aluminium to dispel last traces of chlorine.

17.2.2.4 Fit a 2-hole rubber stopper to flask. Through one hole pass a bulbed glass tube that reaches nearly to surface of liquid. Attach this tube to a large carbon dioxide generator through a short, bulbed tube inserted in second hole of stopper and ending slightly below it. With rubber tube connect this second glass tube to another glass tube, about 25 cm long; immersed in a cylinder of water to a depth of about 20 cm (this connection acts as a seal to restrain any strong flow of gas when not desired and to permit pressure in flask). Raise delivery tube nearly out of water seal, allowing rapid flow of carbon dioxide for few minutes to dispel air from system. Then lower delivery tube into water seal, slightly raise stopper, and quickly drop into flask 1 to 2 g sheet aluminium, folded into narrow bent strip to prevent breaking of flask. After aluminium dissolves completely, raise tube in water seal, letting carbon dioxide pass through rapidly. Place flask on hot plate and boil for a few minutes. Remove flask from heat and cool with tap or ice-water, containing flow of carbon dioxide. Lower delivery tube into cylinder, disconnect flask, and, with glass plug close rubber tube through which carbon dioxide enters flask. Wash glass tubes, rubber stopper and sides of flask with air free wash solution (*see* **17.2.1.1**). Add starch indicator (*see* **17.2.1.5**) and titrate immediately with 0·01 N iodine solution.

17.2.2.5 If desired, carry out titration by slightly raising rubber stopper after cooling and adding excess 0·01 N iodine solution. Then disconnect flask, wash tubes, rubber stopper, and sides of flask with air-free wash solution; and titrate excess iodine with 0·1 N sodium thiosulphate.

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